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Benefits of probiotics in preterm neonates in low and medium income countries - a systematic review of randomised controlled trials

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Benefits of probiotics in preterm neonates in low and medium income countries - a systematic review of randomised controlled trials

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Abstract

Objective: Although there is an overall reduction in under five mortality rate, the progress in reducing neonatal mortality rate has been very slow. Over the last 20 years, preterm births have steadily increased in low and medium income countries (LMIC) particularly in sub-Saharan Africa and South Asia. Preterm birth is associated with increased mortality and morbidity, particularly in LMICs. Based on systematic reviews of randomised controlled trials (RCT), many neonatal units in high income countries have adapted probiotics as standard of care for preterm neonates. Given their simplicity and affordability, we aimed to systematically review the safety and efficacy of probiotics in reducing mortality and morbidity in preterm neonates in LMICs.

Design: Systematic review and meta-analysis of randomised controlled trial

Data sources: Medline, EMBASE, Cochrane Central Register of Controlled Trials, CINAHL (Cumulative Index of Nursing and Allied Health Literature), and E-abstracts from the Pediatric Academic Society meetings and other paediatric and neonatal conference proceedings were searched in January 2017

Eligibility criteria: RCTs comparing probiotics vs. placebo/no probiotic in preterm neonates (gestation <37 weeks) conducted in LMICs.

Results: Total 23 (N=4783) RCTs from 4 continents and 10 LMICs, were eligible for inclusion in the meta-analysis using fixed effects model. The risk of NEC [RR: 0·46(95% CI: 0·34, 0·61) p <0.00001, NNT: 25 [95% CI: 20, 50], late onset sepsis (LOS) [RR: 0·80(95% CI: 0·71, 0·91) p=0.0009, NNT: 25 [95% CI: 17, 100] and death [RR: 0·73(95% CI: 0·59, 0·90) p=0.003, NNT: 50 [95% CI: 25, 100] was significantly lower. The results were significant on random effects model analysis and after excluding studies with high risk of bias. No significant adverse effects were reported.

Conclusion: Probiotics have significant potential to reduce mortality and morbidity (e.g. NEC, LOS) in preterm infants in LMICs

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Strengths and limitations of this study

- To our knowledge this is the first systematic review focusing on RCTs of probiotics in preterm neonates in LMICs.
- Strengths of our review include reliable results, considering the robust methodology, number of trials from different LMICs (23 studies from 4 continents and 10 LMICs), large sample size (N=4783), low/no statistical heterogeneity, and the small p values indicating the low probability of chance.
- Results showing beneficial effects of probiotics in reducing sepsis, NEC and mortality are significant considering the United Nation's MDG4 and UN Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying Every Woman, Every Child initiative, Every Newborn Action plan (ENAP), and the burden of prematurity in LMICs
- Although there was no significant statistical heterogeneity there were variations in types of probiotics used in different studies and limitations of certain study quality

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Introduction

The UNICEF 2010 report showed that the global burden of under five mortality was reduced by one third compared to 1990s; however progress in reducing neonatal mortality has been slow. [1-3] Almost 40% of under five deaths occur during the neonatal period and majority of these deaths occur in Sub-Saharan Africa, South Asia, and Oceania. An estimated 98% of all neonatal deaths occur in low and medium income countries (LMIC). [4-6] Out of 135 million births each year, 3.1 million have died within the neonatal period and nearly 35% of these deaths occur in preterm neonates.[2,5] It may be perceived that prematurity is not a problem of LMICs. However, it is important to note that only 8.6% of preterm births occur in developed countries[5] Over the last 20 years, the number of preterm births has steadily increased to 9.1million as of 2010 in the regions of sub-Saharan Africa and South Asia. Preterm birth is associated with increased risk of mortality, and morbidity including late onset sepsis (LOS), necrotising enterocolitis (NEC), feeding difficulties, and long term neurodevelopmental impairment (NDI). [6-8] Although survival of preterm neonates has improved in some LMICs, morbidities such as NEC and LOS are still a major issue.[5,9-12] Considering the United Nation's millennium developmental goal (MDG-4), and the United Nation Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying 'Every Woman, Every Child initiative, Every Newborn Action plan' (ENAP), it is important to develop cost-effective simple strategies to reduce the mortality and morbidity associated with prematurity in LMICs.[13]

The World Health Organisation (WHO) defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".[14] Probiotics have been shown to significantly reduce the risk of NEC, all-cause mortality, LOS and facilitate feed tolerance in preterm very low birth weight (VLBW) neonates.[15-17] The mechanisms of benefits of probiotics include gut barrier enhancement, immune response

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modulation (e.g. TLR4 receptor, nuclear factor-B, inflammatory cytokines), and direct inhibition of gut colonisation by pathogens.[18-22] Many developed countries are already using probiotics routinely in preterm neonates for prevention of NEC.[23-32] It has been suggested that probiotics may have a role in LMICs for prevention, treatment of acute gastrointestinal diseases, particularly in children with HIV infection.[33-36] Given their simplicity and affordability, we aimed to systematically review the safety and efficacy of probiotics in reducing the risk of mortality and morbidity in preterm neonates in LMICs.

METHODS

Guidelines from the Cochrane Neonatal Review Group (http://neonatal.cochrane.org/resources-review-authors),[37] Centre for Reviews and Dissemination (http://www.york.ac.uk/crd/guidance/),[38] and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [39] were followed for undertaking and reporting this systematic review and meta-analysis. Ethics approval was not required.

Eligibility Criteria:

Types of studies: Only randomised controlled trials (RCTs) were included in the review. Observational studies, narrative/systematic reviews, case reports, letters, editorials and commentaries were excluded, but read to identify potential additional studies.

Types of participants: Preterm neonates born at a gestational age (GA) <37 weeks or low birth weight (LBW: <2500 grams) or both (Same criteria as the Cochrane review, 2014).[15]

Setting: Only RCTs from LMICs were included. LMICs were defined as per the World Bank guidelines which include countries with gross national income (GNI) per capita of under $12736/year.[40]

Intervention and comparison: Enteral administration of probiotic supplement versus control (placebo/no probiotic).

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Outcomes: All-cause mortality, LOS (Positive blood/CSF culture on a sample collected 48-72 hours after birth), Definite NEC (Stage ≥II modified Bell staging)[41] and time to full enteral feeds. (TFF: 120ml/kg/day).

Search strategy: The databases Medline searched via PubMed (www-ncbi-nlm-nih-gov, 1966-2017), EMBASE (Excerpta Medica dataBASE) via Ovid (http://ovidsp.tx.ovid.com, 1980-2017), Cochrane Central Register of Controlled Trials (www.thecochranelibrary.com, through January 2017), CINAHL (Cumulative Index of Nursing and Allied Health Literature) via OVID (http://ovidsp.tx.ovid.com, 1980- January 2017), and E-abstracts from the Pediatric Academic Society meetings (www.abstracts2view.com/pasall, 2000- January 2017) were searched in January 2017. Abstracts of other conference proceedings such as European Academy of Paediatric Societies (EAPS) and the British Maternal and Fetal Medicine Society were searched in EMBASE. 'Google Scholar' was searched for articles that might not have been cited in the standard medical databases. Grey literature was searched using the national technical information services (http://www.ntis.gov/), Open Grey (http://www.opengrey.eu/), and Trove (http://trove.nla.gov.au/). We have also searched LILACS and Caribmed via the BIREME/PAHO/WHO - Latin American and Caribbean Center on Health Sciences Information (http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/) using broad terminologies Probiotics OR Probiotic Or Bifidobacterium OR Bifidobacteria OR Lactobacillus OR Lactobacilli OR Saccharomyces. We also searched https://clinicaltrials.gov, http://www.who.int/ictrp/en/, and www.bioportfolio.com for ongoing RCTs. The reference lists of eligible studies and review articles were searched to identify additional studies. Reviewers SR, GJ and GD conducted the literature search independently. No language restriction was applied. The non-English studies were identified by reading the recent systematic reviews of probiotic supplementation for reducing the risk of NEC,[42,43] and from cross references of individual studies. Full texts of all non-English

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studies were obtained via University of Sydney and Department of New South Wales (NSW) health library. A research officer from the NSW Health, University of Sydney translated the articles. Attempts were made to contact the authors for additional data and clarification of methods. Only published data were used for those studies, where available.

PubMed was searched using the following terminology: ((("Infant, Newborn"[Mesh]) OR ("Infant, Extremely Premature"[Mesh] OR "Infant, Premature"[Mesh])) OR ("Infant, Low Birth Weight"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh])) AND "Probiotics"[Majr]. It was also searched using (("Infant, Extremely Premature"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh] OR "Infant, Small for Gestational Age"[Mesh] OR "Infant, Premature, Diseases"[Mesh] OR "Infant, Premature"[Mesh] OR "Infant, Newborn, Diseases"[Mesh] OR "Infant, Newborn"[Mesh] OR "Infant, Low Birth Weight"[Mesh])) AND ((("Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Saccharomyces"[Mesh]). The other databases were searched using similar terminologies. The detailed search terminology is given in appendix 1.

Study selection: The abstracts of citations obtained from the initial broad search were read independently by reviewers SR, GJ, and GD, to identify potentially eligible studies. Full-text articles of these studies were obtained and assessed for eligibility by reviewers SR, GJ and GD independently, using the predefined eligibility criteria. Differences in opinion were resolved by group discussion to reach consensus. Care was taken to ensure that multiple publications of the same study were excluded to avoid data duplication.

Data extraction: Reviewers GD, SR and GJ extracted the data independently using a data collection form designed for this review. Information about the study design and outcomes was verified by all reviewers. Discrepancies during the data extraction process were resolved by group discussion. We contacted authors for additional information/clarifications.

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Assessment of risk of bias (ROB): ROB was assessed using the Cochrane "Risk of Bias Assessment Tool".[44] Authors GD, SR and GJ independently assessed the ROB in all domains including random number generation, allocation concealment, blinding of intervention and outcome assessors, completeness of follow up, selectivity of reporting and other potential sources of bias. For each domain, the ROB was assessed as low, high or unclear risk based on the Cochrane Collaboration guidelines.

Data synthesis: Meta-analysis was conducted using Review Manager 5•3 (Cochrane Collaboration, Nordic Cochrane Centre). Fixed-effects model (FEM) (Mantel–Haenszel method) was used. Random-effects model (REM) analysis was conducted to ensure that the results and conclusions were not influenced by the type of model used for the meta-analysis. Effect size was expressed as risk ratio (RR) and 95% % confidence interval (CI).

Statistical heterogeneity was assessed by the $\chi 2$ test, I2 statistic and visual inspection of the forest plot (overlap of CIs). A p value <0.1 on $\chi 2$ statistic was considered to indicate heterogeneity. An I^2 statistic values were interpreted as per the Cochrane handbook guidelines as follows: 0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity.[37] The risk of publication bias was assessed by visual inspection of the funnel plot.[45]

Subgroup analysis: a) Low ROB: random sequence generation and allocation concealment b) Premature neonates less than 34 weeks gestation or birth weight less than 1500g.; c) Where *bifidobacterium* was part of the supplementation; d) Where *lactobacillus* was part of the supplementation; e). Single strain probiotic were used and f) Multiple strain probiotics were used.

Summary of findings table: The key information concerning the quality of evidence, the magnitude of effect of the intervention and the sum of available data on the main outcome

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was presented in the 'summary of findings table' as per the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) guidelines.[44]

Funding source: Not required

Results: The literature search retrieved 1926 potential relevant citations. After carefully reviewing the abstracts, 1814 studies were excluded: Reviews: 378, observational studies: 187, commentaries: 49, case reports: 147, RCTs in adult and paediatric population: 53, and non-relevant studies: 982. Finally 23 RCTs (n=4783) conducted in 10 different LMICs in 4 continents were included in the meta-analysis. [12,46-67] The search strategy results are given in appendix 1. The flow diagram of study selection process is given in **figure 1**. The characteristics of the included studies are given in **table 1**. Out of the 23 included studies, Single-strain probiotics were used in 11 studies, whereas 12 used multiple strains. *Lactobacillus* was part of the supplementation in 13 studies; *bifidobacterium* was part of the supplementation in 11 studies and saccharomyces in 3 studies. **(Table 1).**

ROB of included studies: A total of 14/23 (60%) included studies were judged to have low ROB for the domain of 'random sequence generation', and (56%) were considered to have low ROB for 'allocation concealment'. **(Table 2)**

Effect of probiotics on ≥ Stage II (definite) NEC (Figure 2): Data on definite NEC was reported by 20 trials (N=4022).[12,46-53,55,56,58-65,67] A higher proportion of neonates in the control group developed definite NEC compared with the probiotic group [65/2065 (3.1%) vs. 135/1957 (6.9%)]. Meta-analysis using a FEM estimated a lower risk [RR: 0.46 (95% CI: 0.34, 0.61), p<0.00001] of NEC in the probiotic group. There was no significant heterogeneity (I^2 =19%, p=0·22) among the trials. The numbers needed to treat (NNT) with probiotics to prevent one case of NEC was 25 [95% CI: 20, 50].

Effect of probiotics on LOS (Figure 3): Data from 18 trials[12,46,47,49,51-54,56-62,64,65,67] (N=4062) showed that a higher proportion of neonates in the control group developed LOS

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compared with those in the probiotic group [308/2076 (14.5%) vs. 358/1986 (18%)]. Meta-analysis using a FEM estimated a lower risk [RR: 0.80 (95% CI: 0.71, 0.91), p=0.0009] of LOS in the probiotic group. There was no significant heterogeneity (I^2 =25%, p=0.16) among the trials. The NNT with probiotics to prevent one case of LOS was 25 (95% CI: 17, 50).

Effect of probiotics on all cause mortality (Figure 4): Data from 19 trials (N=4196),[12,46-49 51-54 56-65] showed reduced risk of death due to all causes in the probiotic vs. control group [137/2148 (6.37%) vs. 176/2048 (8.59%)] Meta-analysis using a FEM estimated a lower risk [RR: 0.73(95% CI: 0.59, 0.90), p=0.003] of death in the probiotic group. No significant heterogeneity was noted between the trials (I^2 =0%, p=0.67). The NNT to prevent one death by probiotic supplement was 50 (95% CI: 25, 100).

Effect of probiotics on TFF (Figure 5) Meta-analysis of data (N=2154) from 13 trials [12,47-49, 53,56,59-63,65,66] showed significant reduction in TFF in the probiotics vs. control group [MD=-3.09 days (95% CI: -3.49, -2.69), p<0.00001]. However, there was significant heterogeneity (I^2= 90%, p< 0.00001) among the trials. These results were hence checked by using REM and remained significant [MD=-1.95 days (95% CI: -3.44, -0.45), p=0.01].

Subgroup analysis: The beneficial effects continued to be observed in studies a) Low ROB: random sequence generation and allocation concealment **(Table 3)** b). That only included infants with gestational age <34 weeks or birth weight <1500g; c) Where *bifidobacterium* was part of the supplementation; d) Where *lactobacillus* was part of the supplementation; e). Single strain probiotics were used and f) Multiple strain supplements were used; however, on REM meta-analysis, statistical significance was lost for some of these analyses **(Table 4).** The overall evidence according to GRADE guidelines is provided as a summary of findings table **(Table 5)**. The evidence was deemed high in view of the large sample size, low risk of bias in majority (14/20) of the included studies, narrow confidence intervals around the effect

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size estimate, very low p value for effect size estimate and mild statistical heterogeneity. Visual inspection of the funnel plot suggested that there was no publication bias **(Figure 6).**

Safety: None of the studies reported any significant adverse effects including probiotic sepsis.

Discussion:

The results of our systematic review of 23 RCTs (N=4783) conducted in ten LMICs across four continents show that probiotic supplementation in preterm neonates (born <37 weeks) significantly reduces the risk of all-cause mortality, LOS and NEC in such a set up. The limitation of this review include variations in types of probiotics used in different studies and limitations of study qualities in few studies. The strengths of our review include reliable results, considering the robust methodology, number of trials from different LMICs, large sample size, low/no statistical heterogeneity, and the small p values indicating the low probability of chance. Summary findings as per GRADE guidelines confirm the high quality evidence (Table 5). To our knowledge this is the first systematic review focusing on RCTs of probiotics in preterm neonates in LMICs. Our results are significant considering the United Nation's MDG4 and UN Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying Every Woman, Every Child initiative, Every Newborn Action plan (ENAP), and the burden of prematurity in LMICs.[4,5,13]

The incidence of prematurity is significantly increasing in LMICs compared to Europe or North America. There are issues related to reporting of preterm births and outcomes in LMICs.[68] However the studies funded by the WHO estimate 13 million preterm births/year in LMICs with 11 million (85%) of these being concentrated in Africa and Asia, ~0.5 million each in Europe and North America (excluding Mexico) and 0.9 million in Latin America and the Caribbean.[69] The highest rates (11.9%) and number (seven million) of preterm births were in Africa, and Asia respectively. Mortality and morbidities such as LOS, NEC and feeding

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difficulties are major issues in preterm neonates. Although specific data from LMICs is not available, ~one million preterm neonates die every year, predominantly due to sepsis, and long-term impairment in survivors is becoming an important issue. [70]

Consistent with our recent systematic review[71], our results show that probiotics reduced the risk of not only NEC and all-cause mortality but also of LOS in preterm neonates. [RR: 0.81 (95% CI: 0.71, 0.92), p=0.001]. The reduction of LOS by probiotics is important considering that neonatal sepsis is responsible for nearly a 3rd all neonatal deaths in LMICs.[19, 20,22,72-77]

It is important to note that the burden of NEC is as significant in LMICs as in high income countries. The incidence and severity of NEC is higher in LMICs and includes up to 15% cases of NEC totalis with ~100% mortality.[9,12] It occurs not only in VLBW and ELBW neonates but also in preterm neonates with higher birth weight. Lack of antenatal steroids and being small for gestational age (SGA) due to intrauterine growth restriction (IUGR) are known risk factors for NEC.[78] The reason for higher incidence of NEC in LMICs could include the higher numbers of preterm 'SGA-IUGR' births and limited coverage of antenatal steroids.[79,80] The NEC related mortality and morbidity is almost entirely due to progression of the illness from stage II to stage III. Management of surgical NEC is difficult in LMIC considering the limited resources. Primary prevention of NEC is therefore an important strategy for reducing the health burden of the condition in LMICs. Considering the effect size with regards to reduced risk of NEC, the benefits of probiotics in LMIC could not be overemphasised.

The issue of implementing probiotics for preterm neonates in LMICs is complex. The options include either reconfirming their safety and efficacy in large definitive RCTs in LMICs or adopting their routine use based on current evidence. Conducting large multicentre trials and accessing proven safe and effective probiotics is difficult, especially in resource limited set ups.[34] Apart from the significant budget the difficulties include regulatory hurdles, and

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logistics of importing a probiotic product, maintaining cold chain, and providing ongoing independent safety and quality control. However there are recent examples of large RCTs conducted successfully in community settings in LMICs.[81,82] Neonatal demographic characteristics such as gestation and IUGR, are an important issue in conducting RCTs in LMICs as they determine the risk of NEC, duration of probiotic supplementation, and the cost-benefit ratio. It is also important to note that many RCTs have used different probiotic/s and probiotic activity could be strain specific.

Knowledge of the pattern of gut colonisation in preterm neonates in a given set up is important before using probiotics for research or routine use. Dutta et al have reported abnormal intestinal colonization patterns in the first week of life in VLBW neonates in their level III neonatal intensive care unit in India.[52] On day one, 45% neonates had sterile guts, and by day three, all were colonized predominantly by E. coli, K. pneumoniae and Enterococcus fecalis. Only one isolate had *lactobacilli* and *bifidobacteria* were not detected during the study period. Formula feeding was associated with E. coli colonization. Results of completed [82] and ongoing trials such as NCT02552706 will be important.[83]

Probiotic sepsis, antibiotic resistance, and altered immune responses in the long run, are the potential adverse effects of probiotics in preterm neonates. Availability of killed or inactivated probiotic strains with clinically proven benefits may help not only in avoiding such adverse effects but also in avoiding the need to maintain the cold chain. Awad et al have compared the effect of oral killed (KP) versus living *lactobacillus acidophilus* (LP) in reducing the incidence of LOS and NEC in neonates.[46] Both LP and KP reduced the risk of NEC [Absolute risk reduction (ARR): 16%, 15%, respectively] and LOS (ARR: 18%) significantly compared with placebo. LOS and NEC was reduced significantly in neonates colonised versus not colonised by *lactobacillus* at day 7 (27.9 vs. 85.9%, 0 vs. 7.8%) and day 14 (48.7 vs. 91.7% for LOS and 0 vs. 20.8% for NEC). KP retained the benefits similar to LP

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on comparison between all groups. Given the global implications of these results, the benefits of inactivated/killed probiotics need to be assessed in further large definitive trials.

In summary our results indicate that probiotics are effective in significantly reducing the risk of all-cause mortality, LOS, and NEC in preterm VLBW neonates in LMICs. Considering the burden of death, disease (NEC, LOS), and suboptimal nutrition in preterm neonates in LMICs, cooperation between various stake holders (e.g. industry, scientists, regulatory agencies) is warranted to either develop or to improve access to high quality safe and effective probiotics in such set ups. Support from organisations such as the WHO is important in providing access to probiotics for the countries (e.g. sub-Saharan Africa) where most prematurity related deaths occur. Whether probiotics could be used for research and/or routine use in preterm neonates in LMICs will depend on the national health priorities, resources, and ethics.

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Authors' contributions: Dr Deshpande conceptualized and designed the study, performed an independent literature search, selected studies for inclusion, extracted and interpreted data, assessed risk of bias of included studies, handled the meta-analysis software, oversaw translation of manuscripts in the Chinese language and wrote the first and final drafts of the manuscript; Dr Athalye-Jape performed an independent literature search, selected studies for inclusion, contacted authors for additional information where necessary, extracted and interpreted the data, checked the data entered by Dr Deshpande on the meta-analysis software, assessed the risk of bias of included studies, and helped with the first and the final draft of the manuscript; Dr Rao performed an independent literature search, selected studies

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for inclusion, verified the extracted data, assessed risk of bias, interpreted data, and helped with the first and the final draft of the manuscript; Prof Patole supervised the project, acted as referee author in case of differences of opinion between the first 3 authors, interpreted the data, and supervised the first and approved the final versions of the manuscript; and all authors approved the final manuscript as submitted.

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Table 1: **Characteristics of included studies**

Study ID/	Location	Study characteristics
1. Awad 2010[40]	**Egypt**	**Participants:** All neonates admitted to nursery, 28-41 weeks and weight 1.1-4.3 kg **Intervention and dose:** Killed probiotic/KP (*L. acidophilus,* 6× 10^9 CFU) vs. Living probiotic/LP (*L. acidophilus,* 6× 10^9 CFU) vs. Placebo **Duration of supplementation:** commenced on D1, duration NA **N=150** (60 vs. 60 vs.30), **Preterm: 89** (37 vs. 36 vs. 16) **Type of milk:** details NA **Type of delivery:** Preterm CS: KP (57%) vs. LP (56%) vs. Placebo (75%) **Primary outcome: All outcomes for LP vs. KP vs. Controls:** Incidence of neonatal sepsis (18/36, 50% vs. 25/37,68% vs. 12/16, 75%; p=0.251) and NEC (0/36 vs. 1/37 vs.5/16; p=0.000) neonates and evaluation of efficacy of a KP **Other outcome: Mortality:** 4/36 (11.1%) vs. 12/37(32.4%) vs. 5/16(31.3%) p=0.076
2. Braga 2010[41]	**Brazil**	**Participants:** Preterm infants 750-1499g **Intervention and dose:** (L. *casei* + B. *breve* : 3.5x10^7 to 3.5x10^9 CFU) vs. No probiotic **Duration of supplementation::** Once daily from the second day of life until day 30 **N =231** (Probiotics: 119, Controls: 112) **Type of milk**: EBM/ PDHM **Type of delivery**: CS 53.8% vs. 49.1 % **Primary outcome:** ≥ Stage II NEC (0/119, 0% vs. 4/112, 3.6%) **Other outcomes: LOS:** 40/119 (33.6% vs 42/112 (37.5%)**, Mortality:** 26/119 (21.8%) vs. 27/112(24.1%)
3. Dashti 2014[42]	**Iran**	**Participants:** Preterm infants 700-1800 g **Intervention and dose:** (*L. acidophilus*, *L. rhamnosus*, *B. longum*, *L. bulgaricus*, *L. casei*, *S. thermophilus*, *B. breve,* and *Bifidobacterium*: total 1x10^9 CFU/sachet) vs. placebo powder **Duration of supplementation::** Once daily from first feed of life until discharge **N =136** (Probiotics: 69, Controls:67) **Type of milk**: EBM/ formula milk **Type of delivery**: CS 82.4% vs. 17.6 % **Primary outcome:** ≥ Stage II NEC (2/69, 2.9% vs. 1/67, 1.5%) **Other outcomes: Mortality:** 8/69 (11.6%) vs. 4/67(5.97%)
4. Demirel, Erdeve 2013[43]	**Turkey**	**Participants:** Preterm infants ≤32 w and ≤1500g **Intervention and dose: :** S. *boulardii,* 5x10^9CFU vs. no probiotic **Duration of supplementation:** NA **N=271** (Probiotic: 135, Controls: 136) **Type of milk**: EBM/ Formula **Type of delivery**: CS 77.7% vs. 83% **Primary outcome:** NEC ≥ stage 2(6/135, 4.4% vs. 7/136, 5.1%) p=1, **Mortality:**(5/135,3.7% vs. 5/136, 3.7%) p=1 **Other outcomes: LOS:** 20/135 (14.9%) vs 21/136 (15.4%) p=0.906**, Feed intolerance:**30/135(22.2%) vs. 62/136(46%), p<0.001
5. Deng 2010[44]	**China**	**Participants:** 125 preterm infants, <37 weeks, <2500 g at birth **Intervention and dose:** *B. longum, L. acidophilus, Enterococcus fecalis*, triple viable powder oral or nasal Bifico plus powder / capsules. For birth weight <1500 g: 0.33× 10^7 CFU of each probiotic twice daily and >1500 g: 0.5× 10^7 of each probiotic twice daily, Control: sterile warm water **Duration of supplementation:** commenced from first feed till 14 days of life

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		N=125 (**62** Controls 33.2 ± 2.3 weeks vs **63** Probiotic group 32.4 ± 2.8 weeks), **Type of Milk:** EBM/preterm formula **Type of Delivery:** NA **Primary outcome: NEC: Controls**: Bell Stage I (1/62, 1.6%), Bell Stage II (4/62, 6.5%), Bell Stage III (4/62, 6.5%) vs T**reatment** Bell Stage I (1/63,1.6%), Bell Stage II (1/63, 1.6%) **Other outcomes: LOS, Mortality:** NA
6. Dilli 2015[45]	**Turkey**	**Participants:** VLBW infants with a gestation of <32w and birth weight<1500g **Intervention and dose:** B. *lactis* ($5x10^9$ CFU) vs. Placebo (maltodextrin) **Duration of supplementation:** From day 8 of life, once daily until discharge or a maximum of 8 weeks **N=200** (Probiotic 100, Placebo: 100) **Type of milk:** EBM/Formula **Type of delivery: CS**: 35/100 (35%) vs. 37/100 (37%) **Primary outcome: NEC (≥stage 2):**2/100(2%) vs.18/100 (18%), p<0.001 **Other outcomes: LOS:** 8/100 (8%) vs 13/100 (13%), p=0.6, Mortality: 3/100 (3%) vs. 12/100 (12%), p=0.003, Time to full enteral feeds^ (150ml/kg/day): 18(14-23) days vs. 25(15-37) days, p<0.001
7. Dutta 2015[46]	**India**	**Participants:** Preterm infants 27-33 weeks gestation **Intervention:** High dose (10 billion CFU: *L. acidophilus, L. rhamnosus, B. longum, S. boulardii*) **vs.** Low dose (1 billion CFU*: L. acidophilus, L. rhamnosus, B. longum, S. boulardii*) vs. Placebo (potato starch, maltodextrin) **Duration of supplementation: Probiotic groups: (A)**: High dose for 21 days, (C): Low dose for 21 days, (B): High dose short course (D1-D14 and D15-D21) **N: Probiotic (114)** vs. **Placebo (35)** **Type of milk:** EBM /formula **Type of delivery:** Probiotic group vs. Placebo: SVD (69% vs. 60%), CS: data NA **Primary outcome: Stool colonisation rates on D14, D21, D28 with 3 different probiotic regimens** (Lactobacillus and Bifidobacterium colonisation was significantly higher in groups A, B, and C vs. placebo respectively. Groups A, B, and C did not differ from each other. There were trends toward more CFU of Lactobacillus and Bifidobacterium per ml of stool in group A vs. B and B vs. C. Groups A and B and spontaneous preterm labor (SPL) independently predicted high Lactobacillus counts on day 28; groups A, B, and C and SPL predicted high Bifidobacterium counts) **Other outcomes: LOS:** 10/114 (8.8%) vs. 6/35 (17.1%), p=0.14, **Mortality**:8/114(7%) vs.2/35(12.7%), p=0.85, **NEC (≥stage 2):** 6/114(5.3%) vs 0/35(0%), p=0.35
8. Fernandez-Carrocera 2013[47]	**Mexico**	**Participants:** Preterm infants<1500g **Intervention and dosage:** Multispecies probiotic product **(**L. *acidophilus* +L. *rhamnosus* +L. *casei*+ L. *plantarum*+ B. *infantis*+ S. *thermophilus)* vs. No probiotic **Duration of supplementation:** From the day of commencement of enteral feeds, once daily. Actual Duration: NA **N=150** (Probiotics:75, Controls: 75) **Type of milk:** EBM/ Formula **Type of delivery:** data not available **Primary outcome:**≥ Stage 2 NEC: 6/75(8%) vs 12/75(16%), p=0.142 **Other outcomes: LOS:** 42/75 (56%) vs 44/75 (58.7%), p=NA, **Mortality:** 1/75(1.3%) vs. 7/75(9.3%), p=0.063
9. Hua 2014[48]	**China**	**Participants:** Preterm infants<37 weeks **Intervention and dosage:** Probiotic Jin Shuang QI (*L. acidophilus, S. thermophilus, Bifidobacterium*) 5 x 10^7 CFU/day. Vs no probiotic **Duration of supplementation:** From the day of commencement of enteral feeds, once daily. Duration of Supplementation: not clear **N=257** (Probiotics:119, Controls: 138)

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		Type of milk: EBM/ Formula **Type of delivery**: CS 55.5% vs 64.5% **Primary outcome: Stool colonisation by drug resistant bacteria** (No difference in both groups, p>0.05) **Other outcome: LOS:** 2/119(1.7%) vs. 8/138 (5.8%); p-0.168, **NEC (stage NS):** 0/119 vs. 2/138; p=0.501,**Mortality:** 2/119 vs. 2/138
10. Huang 2009[49]	**China**	**Participants:** Preterm infants 28-32 weeks and <1500g **Intervention and dosage:** *Bifidobacterium* (50 million live bacteria/capsule) 0.25x10^8 live bacteria oral/nasally twice daily vs. non-treatment (control) **Duration of supplementation:** From 7 days till 14 days of age **N=183** (Probiotic: 95, Control: 88) **Type of milk:** Not stated **Type of Delivery:** NA **Primary outcomes: NEC:** 2/95 (2.1%), both Bell's stage 1 vs. 9/88 (10.23%): Bell's stage 1:6, stage 2:2, stage 3:1 (p<0.01), Body mass changes/**Weight gain*:** Probiotic group: 8.109 ± 2. 127 g vs. Control group 6. 489 ± 2. 327 g (p<0.01) **Other outcomes: LOS, Death, TFF:** NA**, gut colonisation:** Post 7d of treatment, the two groups' intestinal bacteria and bacteria ratio of the total number of cocci and rods, the differences were statistically significant (P <0. 01). Rod bacteria ratio before and after preventive treatment groups showed no significant difference (P> 0 05.); in the control group rod bacteria ratio difference was statistically significant (P <0. 01)
11. Oncel 2014[50]	**Turkey**	**Participants:** Preterm Infants≤32w and <1500g **Intervention and dosage:** L. *reuteri DSM 17938* in oil based suspension,1x10^8 CFU/day vs Placebo (Oil based suspension without probiotics) **Duration of supplementation:** From the time of first enteral feeds until discharge **N=400** (Probiotics: 200, Placebo: 200) **Type of milk:** EBM/Preterm formula, **Type of delivery**: CS 75% vs. 76% **Primary outcome: Probiotics vs. Controls: ≥ Stage 2 NEC or death:** 20/200(10%) vs. 27/200(13.5%);p=0.27, **NEC (≥stage 2):**8/200(4%) vs.10/200(5%);p=0.63 **Other outcomes: Late Onset Sepsis:** 13/200 (6.5%) vs 25/200 (12.5%);p=0.041, **Time to full feeds***:9.1±3.2 vs. 10.1±4.3 days; p=0.006, **Hospital stay^:**38(10-131) vs 46(10-180) days; p=0.022**, Feed intolerance:**56/200(28%) vs. 79/200(39.5%);p=0.015
12.Qiao 2012[51]	**China**	**Participants:** Preterm 28-34 weeks GA, >1000 g, <72 h life **Intervention:** Bifidobacterium, Lactobacillus, Streptococcus thermophiles, 0.5g per bag **Duration of supplementation:** 0.5 bag three times daily for 3 days after admission to hospital **N=287** (Probiotic: 149 vs Control 138) **Type of milk:** Not stated **Type of Delivery:** No Stats on CS/type of delivery **Primary outcomes:** Time to full oral feeds (7.3 d vs 16.9 d); p<0.05, time to full enteral nutrition (9.8 d vs 16.9 d); p<0.05, LOS (6.7% vs 15.2%); p<0.05, NEC (3.4% vs 10.9%); p<0.05, hospitalisation time (25.0 d vs 30.8 d); p:NA, Mortality*: (6.0 ± 4.0)% and (9.0 ± 6. 5)%;p>0.05
13. Rojas 2012[52]	**Columbia**	**Participants:** Preterm Infants≤2000g **Intervention and dosage:** L. *reuteri DSM 17938*, 1x10^8 CFU, once daily vs Placebo (Oil based suspension without probiotics) **Duration of supplementation:** Commenced within 48 hours of life. Duration: NA **N=750** (Probiotics:372, Placebo:378) **Type of milk:** EBM/Formula **Type of delivery**: VD non-instrumental: 16% (Study) vs. 17% (Placebo), VD instrumental: 0% (Study) vs. 0.5% (Placebo), Elective CS: 18% (Study) vs. 17% (Placebo), Non Elective CS 65% (Study) vs. 65% (Placebo)

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		Primary outcome: Nosocomial infection and mortality:57/372(15.3%) vs. 67/378(17.7%);p=0.38**, Death:** 22/372(5.9%) vs. 28/378(7.4%);p=0.41 **Other outcomes: LOS:** 24/372 (6.5%) vs 17/378 (4.5%);p=0.24, **Duration of hospitalisation**^: 20(11-33) vs. 20(11-38) days; p=0.53
14. Roy 2014[53]	**India**	**Participants:** Preterm infants<37w and birth weight<2500g **Intervention and dosage:** Half of the one gram sachet that contained L. *acidophilus* 1.25x10^9 + B. *longum* 0.125x10^9 + B. *bifidum* 0.125x10^9+ B. *lactis* 1x10^9 vs. Sterile water **Duration of supplementation**: Commenced within 72 hours of birth for six weeks or until discharge **N=112** (Probiotics: 56, Placebo: 56) **Type of milk**: EBM **Type of delivery**: CS 83.9% vs. 76.8% **Primary outcome**: **Enteric fungal colonisation*:** 3.03± 2.33 ×10^5 CFU vs. 3± 1.5×10^5; p=0.03 and **LOS (bacterial and fungal):** 31/56(55.4%) vs. 42/56(75%); p=0.02 **Other outcome: TFEF*:**11.22± 5.04 vs. 15.41± 8.07 days; p=0.016
15.Saengtawesin 2014[54]	**Thailand**	**Participants:** Preterm(<34 w) and VLBW (<1500g) infants **Intervention and dosage:** Probiotic mixture [*L. acidophilus+B. bifidum* each 1 x10^9 CFU/250mg], 125mg/kg twice daily vs. No probiotic **Duration of supplementation:** NA **N=60** (Probiotics: 31, Controls:29) **Type of milk:** EBM/preterm formula **Type of Delivery:** CS 67.7% vs. 62% **Primary outcome: NEC≥ stage 2:** 1 (3.2%) vs 1 (3.4%); p=0.74 **Other outcomes: LOS:** 2(6.45%) vs. 1(3.44%); p=0.53, **TFEF*:** 12.03 ± 5.49 days vs. 13.76 ± 8.25 days (p = 0.64).
16. Samanta 2009[12]	**India**	**Participants:** Preterm(<32 w) and VLBW (<1500g) infants **Intervention and dosage:** Probiotic mixture [*B. infantis* + *B. bifidum* + *B. longum* + *L. acidophilus*, each 2.5x10^9 CFU], administered twice daily vs. No probiotic **Duration of supplementation:** NA **N=186** (Probiotics: 91, Controls: 95) **Type of milk:** EBM **Type of Delivery:** CS 46.15% vs 49.47% **Primary outcomes: Incidence of NEC(≥stage 2):**5/91(1.1%) vs.15/95(15.8%);p=0.042**, Death due to NEC:** Overall death: 4/91(4.4%) vs. 14/95(14.7%); p=0.032**, Feed tolerance: Time to full feeds*:** 13.76 ± 2.28 vs. 19.2 ± 2.02 days; p<0.001 **Other outcomes: LOS:** 13/91 **(**14.3%) vs. 28/95 (29.5%);p=0.02, **Hospital stay*:** 17.17± 3.23 vs.24.07 ±4 days; p<0.001
17. Sari 2011[55]	**Turkey**	**Participants:** Preterm infants<33w or birth weight<1500g **Intervention and dosage:** L. *sporogenes,* 0.35x10^9 CFU, once a day vs. No Probiotic **Duration of supplementation:** From first enteral feed until discharge **N=221** (Probiotics: 110, Controls: 111) **Type of milk**: EBM/ Formula **Type of delivery**: CS 67.3% vs. 75.7% **Primary outcomes: NEC ≥ Stage II:**6/110(5.5%) vs. 10/111(9%); p=0.447, **Death/NEC:** 9/110(8.2%) vs. 13/111(11.7%); p=0.515 **Other outcomes: LOS:** 29/110 (26.4%) vs 26/111 (23.4%);p=0.613, **Hospital stay**: 34.5 vs. 30 days; p=0.919, **Time to full feeds***: 17.3±8.7 vs. 18.3±9.8 days, p=0.438, **Feed intolerance:** 49/110(44.5%) vs. 70/111(63.1%);p=0.006
18. Serce 2013[56]	**Turkey**	**Participants:** Preterm infants<32 weeks and <1500g **Intervention and dosage:** S. *boulardii* 0.5x10^9 CFU twice daily vs. Placebo (Distilled Water)

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		Duration of supplementation: From the first enteral feed until discharge **N=208** (Probiotic: 104, Placebo: 104) **Type of milk:** EBM/ Formula **Type of Delivery:** CS 80.8% vs 88.5% **Primary outcomes: Stage ≥ 2 NEC:** 7/104(6.7%) vs. 7/104(6.7%); p=1, **LOS:** 19/104 (18.3%) vs. 25/104 (24.3%);p=0.29 **Other outcomes: Death:** 5/104(4.8%) vs. 4/104(3.8%);p=0.74, **Hospital stay^:** 39(28-60) days vs. 43(29-60) days; p=0.62
19. Shadkam 2015[57]	**Iran**	**Participants:** Preterm infants 28 to 32 weeks and 1000-1800g **Intervention and dose:** (*L. reuteri DSM 17938*.: $2.x10^7$ CFU) vs. distilled water **Duration of supplementation::** Twice daily started once infant reached 40ml/kg/day of feed till 120ml/kg/day of feed **N =60** (Probiotics: 30, Controls: 30) **Type of milk**: EBM/ formula milk **Type of delivery**: details NA **Primary outcome: (**Stage NS) NEC (2/30, 6.7% vs. 11/30, 36.7%); p=0.005 **Other outcomes: LOS:** 4/30(13.3%) vs. 10/30(33.4%); p=0.109**, TFEF*:** 12.83±4.26 vs. 16.78±6.66 days; p=0.01, **Mortality:** 1/30 (3.3%) vs. 2/30(6.7%); p=0.5
20.Tewari 2015[58]	**India**	**Participants:** Preterm infants <34 weeks (2 groups: extremely preterm/EPT: 27-30+6weeks and Very preterm/VPT: 31-33+6 weeks) **Intervention:** *Bacillus clausii* (2.4×10^9 spores per day) vs. Placebo **Duration of supplementation:** Commenced D5 in asymptomatic and D10 in symptomatic neonates and continued for 6 weeks/discharge/death/occurrence of LOS whichever was earlier **N=244** (Study: EPT: 61 and VPT:62) vs.(Placebo:121) **Type of milk:** EBM/PDHM **Type of delivery:** CS: EPT: 66% vs 59% and VPT: 58% vs. 60% **Primary outcome: Incidence of definite and probable LOS: Definite LOS:** EPT: 6/61(10%) vs. 8/59(14%); p=0.26, VPT: 2/62(3%) vs. 3/62(5%);p=0.39, **Probable LOS:** EPT: 8/61(12%) vs. 9/59(15%), VPT: 4/62(6%) vs. 5/62(7%) **Other outcomes: Death:** EPT: 8/61(13%) vs. 9/59(15%);p=0.84, VPT: 4/62(7%) vs. 5/62(8%);p=0.79, **NEC (≥stage 2):** EPT: 0/61 vs. 0/59, VPT: 0/62 vs. 0/62
21. Van Niekerk 2015[59]	**S. Africa**	**Participants:** Preterm infants<34 weeks and birth weight 500g to 1250g **Intervention and dosage:** Pro-52 **(***L. rhamnosus GG and B. infantis*) ,$0.35x10^9$ CFU of each daily vs. Placebo (MCT oil) **Duration of supplementation:** From the first enteral feed till day 28 of life **N=184** (Probiotic: 91, Placebo: 93) **Type of milk:** EBM/ Formula **Type of Delivery:** CS 80.8% vs 88.5% **Primary outcome:** Impact of probiotic supplementation on the incidence and severity of NEC in premature VLBW infants that are exposed to HIV. **NEC:** 3/91(3.3%) vs 6/93(6.45%) **Other outcomes: LOS:** 15/91(16.5%) vs 10/93(10.8%)**, Death:** 5/91(5.5%) vs 6/93(6.45%), **TFEF*: HIV exposed:** 10.19±4.055 vs. 9.68 ± 3.46 days, p=0.56 and **HIV non-exposed:** 9.63± 2.42 vs. 11.14± 4.15 days, p=0.022
22.Yang 2011[60]	**China**	**Participants:** 62 preterm infants <37 weeks **Intervention:** *B. longum, L. acidophilus, Enterococcus fecalis* triple viable powder oral or nasal Bifico plus powder / capsules (probiotics powder / capsules), Shanghai Xinyi Pharmaceutical Co., Ltd.), 0.5×10^7 CFU twice daily of each **Duration of supplementation:** from commencement of feeds till 14 days of life **N=62** (Controls:31, Probiotics: 31) **Type of milk:** EBM/preterm formula **Type of Delivery:** NA

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		Primary outcomes: NEC incidence: 2/31 (6.45%) vs. 3/31 (9.68%) vs (No mention of criteria for NEC used) **Other outcomes: Sepsis, Mortality, TFEF:** NA
23. Xu 2015[62]	**China**	**Participants:** 125 neonates with a gestational age of 30-37 weeks and birth weight 1500-2500 g. **Intervention:** *S. boulardii* CNCM I-745 at a dose of 50 mg/kg (10^9 CFU) twice a day **Duration of supplementation:** 9-28 days (mean 25.3 days) **N=125** (Probiotic:63, Control:62), Analysis (Probiotic:51, Control:49) **Type of milk:** EBM/formula **Type of delivery:** NA **Primary outcome:** Weight gain was 16.14 ± 1.96 g/kg/day vs. 10.73 ± 1.77 g/kg/day; p<0.05 and Linear growth was 0.89 ± 0.04 cm/week vs 0.87 ± 0.04 cm/week; p=0.17 **Other outcome:** TFEF: 0.37 ± 0.13 vs 1.70 ± 0.45; p<0.01, maximal enteral feeding volume tolerated :128.44 ± 6.67 vs. 112.29 ± 7.24 ml/kg/day: p=0.03, and duration of hospitalization: 23.3 ± 1.6 vs. 28.0 ± 1.8; p=0.035

Abbreviations: L: Lactobacillus, B: Bifidobacterium, S: Saccharomyces, CFU: Colony Forming Unit, VLBW: Very Low Birth Weight, CS: *Caesarean section,* ***EBM:*** *expressed breast Milk,* ***EOS:*** *early onset sepsis,* ***EPT:*** *Extremely preterm,* ***LGG:*** *Lactobacillus rhamnosus GG (ATCC 53103) Gorbach and Goldin,* ***LOS:*** *Late onset sepsis,* ***LS-*** *Lower Segment,* ***LSCS:*** *Lower Segment Caesarean Section,* ***NA:*** *Data Not Available,* ***NEC:*** *Necrotizing enterocolitis,* ***NS:*** *not specified,* ***PDHM:*** *pasteurised donor human Milk,* ***PMA:*** *post menstrual age,* ***SCFA:*** *Short Chain Fatty Acid,* ***TFEF:*** *Time to full enteral feeds,* ***VD:*** *Vaginal delivery,* ***VPT:*** *very preterm*

- ***For all outcomes, results in the study/probiotic group are given first***
- ***^: median and interquartile range (25-75%), * : mean and SD***

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Table 2: Risk of Bias of the included RCTs

Author/ Year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Awad 2010	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Braga 2010	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dashti 2015	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Demirel 2013	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Deng 2010	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Dilli 2015	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dutta 2015	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Fernandez-Carrocera 2013	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hua 2014	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk
Huang 2009	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Oncel and Sari 2014	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Qiao 2012	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Rojas 2012	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Roy 2014	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Saengtawesin 2014	Low risk	Unclear risk	High risk	High risk	Low risk	Low risk	Unclear risk
Samanta 2008	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
Sari 2011	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shadkam 2015	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Serce 2013	Low risk	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Tewari 2015	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Van Niekerk 2014	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Yang 2011	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Xu 2015	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk

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Table 3: Results of the subgroup analysis (risk of bias)

Item	Number of studies	Sample size	RR (95% CI) (FEM)	RR (95% CI) (REM)	I^2 statistic
Definite NEC: Studies with low ROB on random sequence generation	14	3464	0.55 (0.40,0.74)	0.58 (0.42,0.81)	1%
Definite NEC: Studies with low ROB on allocation concealment	13	3035	0.48 (0.34, 0.66)	0.52 (0.33, 0.80)	29%
LOS: Studies with low ROB on random sequence generation	15	3466	0.85 (0.74, 0.97)	0.84 (0.72, 0.98)	18%
LOS: Studies with low ROB on allocation concealment	11	2839	0.86 (0.75, 0.99)	0.85 (0.74, 0.97)	6%
All cause mortality: Studies with low ROB on random sequence generation	14	3366	0.72 (0.57, 0.91)	0.75 (0.60, 0.95)	0%
All cause mortality: Studies with low ROB on allocation concealment	13	3073	0.76 (0.60, 0.96)	0.78 (0.62, 0.99)	0%

LOS: Late onset sepsis; RR: Relative Risk; CI: Confidence Interval; FEM: Fixed effects model; REM: Random effects model; ROB: Risk of bias

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Table 4: Results of the subgroup analysis

Item	Definite NEC			Late onset sepsis			All cause mortality		
	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)
RCTs with gestational age<32 weeks or birth weight<1500g	14 (2886)	0.51(0.37,0.70)	0.56(0.40,0.78)	11 (2470)	0.84(0.71,1.01)	0.84(0.68,1.04)	12 (2591)	0.75(0.61,0.93)	0.78(0.61,0.99)
RCTs: Lactobacillus was part of the supplementation	13 (2595)	0.45(0.32,0.64)	0.48(0.32,0.71)	12 (2979)	0.81(0.70,0.93)	0.79(0.64,0.97)	16 (3473)	0.70(0.56,0.89)	0.73(0.58,0.93)
RCTs: Bifidobacterium was part of the supplementation	11 (1716)	0.35(0.22,0.55)	0.38(0.23,0.63)	9 (1756)	0.76(0.64,0.89)	0.75(0.59,0.94)	12 (2173)	0.70 (0.52,0.93)	0.71 (0.49,1.03)
Single strain probiotic supplementation	11 (2727)	0.46(0.32,0.66)	0.46(0.32,0.66)	9 (2446)	0.86 (0.7,1.04)	0.83(0.67,1.03)	9 (2444)	0.70(0.52,0.94)	0.71 (0.53,0.96)
Multi strain probiotic supplementation	9 (1333)	0.45(0.28,0.73)	0.47(0.28,0.78)	8 (1556)	0.76(0.65,0.90)	0.75(0.59,0.96)	10 (1752)	0.76(0.56,1.03)	0.78 (0.54,1.13)

LOS: Late onset sepsis; RR: Relative Risk; CI: Confidence Interval; FEM: Fixed effects model; REM: Random effects model; ROB: Risk of bias

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Table 5: Summary of findings as per GRADE guidelines[38]

Outcome	Absolute risk					
	Estimate without probiotic supplementation	Corresponding risk estimate with probiotic supplementation	Relative effect (RR) 95% CI	Number of participants	Quality of Evidence GRADE	Comment
Late onset sepsis	358/1986 (18%)	308/1986 (14.5%)	0.80(0.71,0.91); p=0.0009, I^2=25%	3902	High	Ref note*
Mortality	176/2048 (8.6%)	137/2148 (6.4%)	0.73 (0.59,0.9); p=0.003, I^2=0%	4196	High	Ref note*
NEC	135/1957 (6.9%)	65/2065 (3.1%)	0.46 (0.34,0.61); p<0.00001, I^2 =19%	4022	High	Ref note*

*__Note:__ The evidence was deemed high in view of the large sample size, low risk of bias in majority (14/20) of the included studies, narrow confidence intervals around the effect size estimate, very low p value for effect size estimate and mild statistical heterogeneity.

Abbreviations: GRADE, grades of recommendation, assessment, development and evaluation; RR, risk ratio, CI: Confidence interval.

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Figure 1; Flow diagram of search strategy and study selection (January 2017)

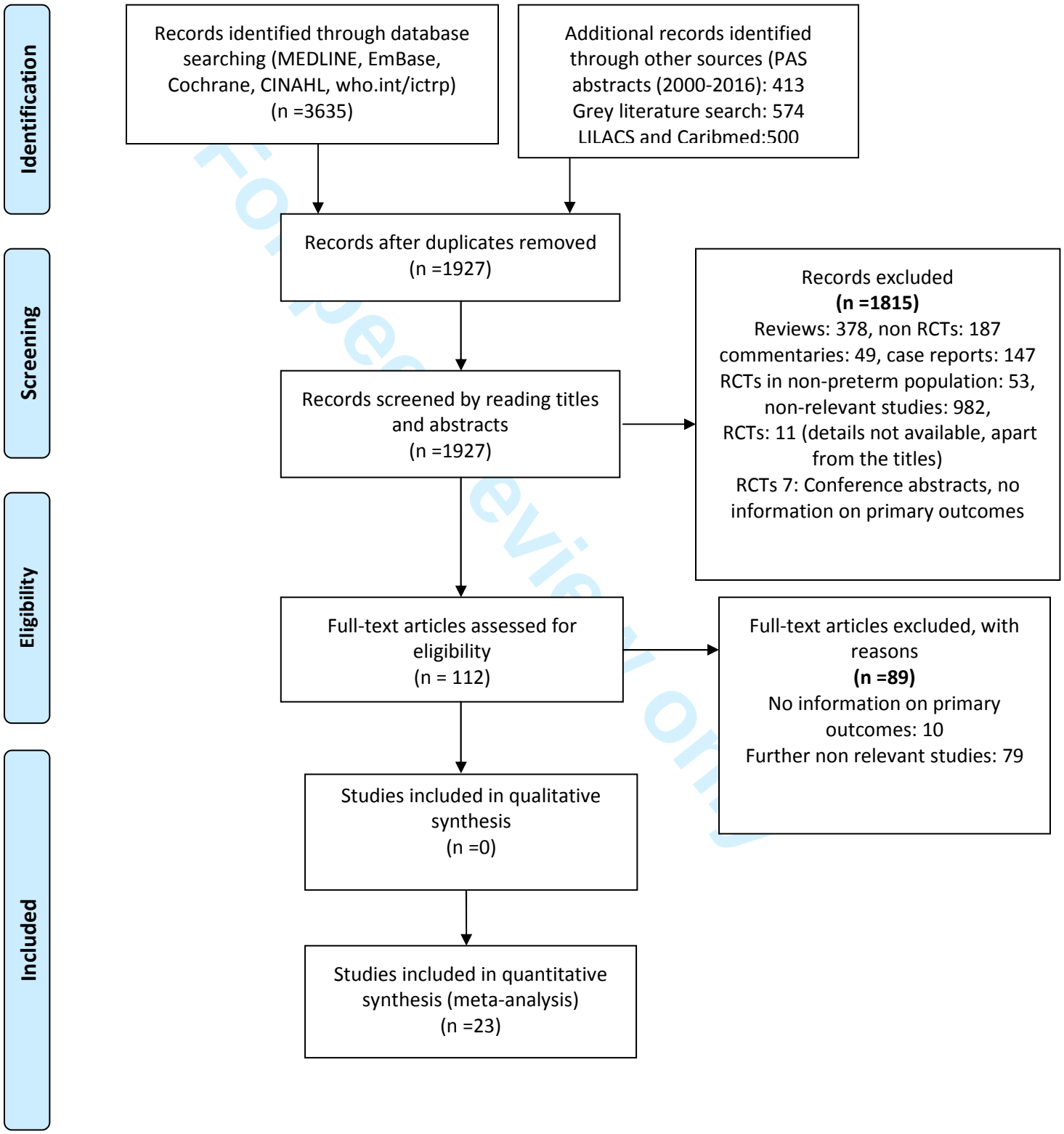

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Figure 2

Forest plot: Effect of probiotics on definite (≥ Stage II) NEC

Study or Subgroup	Probiotic Events	Probiotic Total	No probiotic Events	No probiotic Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Awad 2010	0	36	5	16	5.4%	0.04 [0.00, 0.71]
Braga 2010	0	119	4	112	3.3%	0.10 [0.01, 1.92]
Dashti 2014	1	69	1	67	0.7%	0.97 [0.06, 15.21]
Demirel 2013	6	135	7	136	5.0%	0.86 [0.30, 2.50]
Deng 2010	1	63	8	62	5.8%	0.12 [0.02, 0.95]
Dilli 2015	2	100	18	100	12.9%	0.11 [0.03, 0.47]
Dutta 2015	6	114	0	35	0.5%	4.07 [0.23, 70.49]
Fernandez-Carrocera 2011	6	75	12	75	8.6%	0.50 [0.20, 1.26]
Huang B 2009	0	95	3	88	2.6%	0.13 [0.01, 2.53]
Oncel 2014	8	200	10	200	7.2%	0.80 [0.32, 1.99]
Rojas 2012	9	372	15	378	10.7%	0.61 [0.27, 1.38]
Roy 2014	2	56	2	56	1.4%	1.00 [0.15, 6.85]
Saengtawesin 2014	1	31	1	29	0.7%	0.94 [0.06, 14.27]
Samanta 2008	5	91	15	95	10.6%	0.35 [0.13, 0.92]
Sari 2011	6	110	10	111	7.2%	0.61 [0.23, 1.61]
Serce O 2013	7	104	7	104	5.0%	1.00 [0.36, 2.75]
Shadkam 2015	2	30	11	30	7.9%	0.18 [0.04, 0.75]
Tewari V 2015	0	123	0	121		Not estimable
van Niekerk 2015	3	91	6	93	4.3%	0.51 [0.13, 1.98]
Xu 2016	0	51	0	49		Not estimable
Total (95% CI)		**2065**		**1957**	**100.0%**	**0.46 [0.34, 0.61]**
Total events	65		135			

Heterogeneity: Chi² = 21.07, df = 17 (P = 0.22); I² = 19%

Test for overall effect: Z = 5.34 (P < 0.00001)

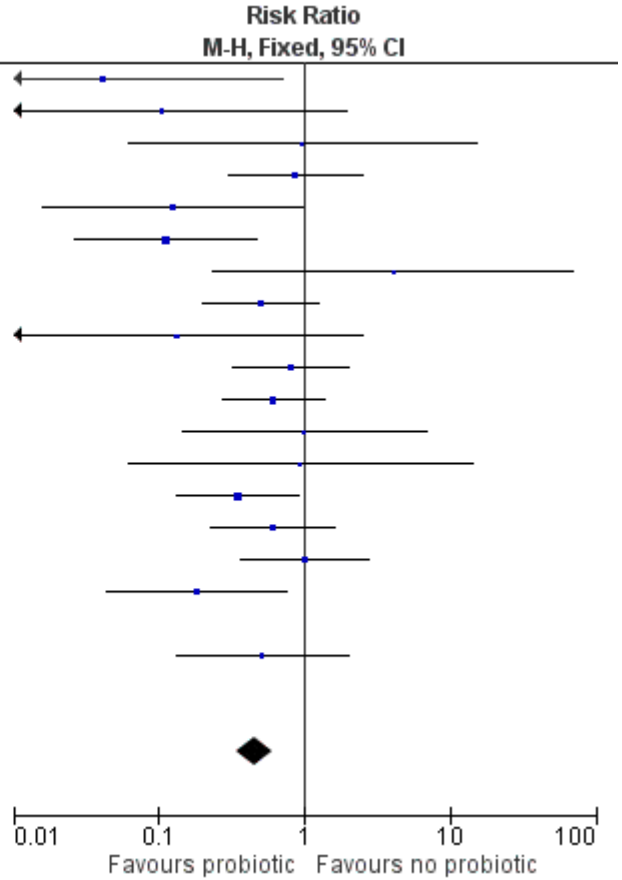

Figure 3

Forest plot: Effect of probiotics on late onset sepsis (LOS)

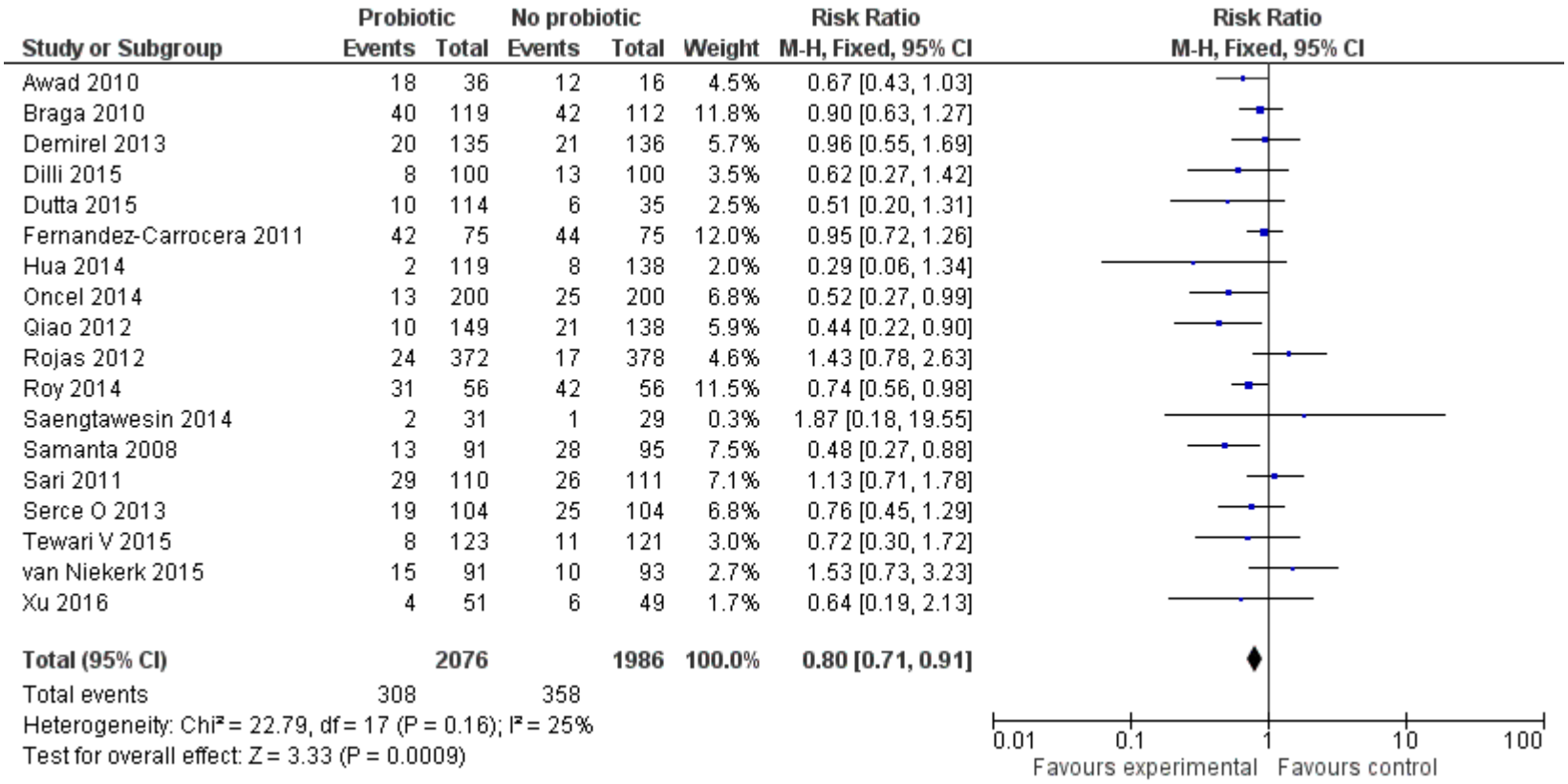

Figure 4

Forest plot: Effect of probiotics on all cause mortality

Study or Subgroup	Probiotic Events	Probiotic Total	No probiotic Events	No probiotic Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Awad 2010	5	60	6	30	4.5%	0.42 [0.14, 1.26]
Braga 2010	26	119	27	112	15.5%	0.91 [0.56, 1.45]
Dashti 2014	8	69	4	67	2.3%	1.94 [0.61, 6.15]
Demirel 2013	5	135	5	136	2.8%	1.01 [0.30, 3.40]
Dilli 2015	3	100	12	100	6.7%	0.25 [0.07, 0.86]
Dutta 2015	8	114	2	35	1.7%	1.23 [0.27, 5.52]
Fernandez-Carrocera 2011	1	75	7	75	3.9%	0.14 [0.02, 1.13]
Hua 2014	2	119	3	138	1.5%	0.77 [0.13, 4.55]
Oncel 2014	15	200	20	200	11.1%	0.75 [0.40, 1.42]
Qiao 2012	6	149	9	138	5.2%	0.62 [0.23, 1.69]
Rojas 2012	22	372	28	378	15.5%	0.80 [0.47, 1.37]
Roy 2014	7	56	8	56	4.5%	0.88 [0.34, 2.25]
Saengtawesin 2014	0	31	0	29		Not estimable
Samanta 2008	4	91	14	95	7.6%	0.30 [0.10, 0.87]
Sari 2011	3	110	4	111	2.2%	0.76 [0.17, 3.30]
Serce O 2013	4	104	5	104	2.8%	0.80 [0.22, 2.90]
Shadkam 2015	1	30	2	30	1.1%	0.50 [0.05, 5.22]
Tewari V 2015	12	123	14	121	7.9%	0.84 [0.41, 1.75]
van Niekerk 2015	5	91	6	93	3.3%	0.85 [0.27, 2.69]
Total (95% CI)		**2148**		**2048**	**100.0%**	**0.73 [0.59, 0.90]**
Total events	137		176			

Heterogeneity: $Chi^2 = 13.98$, $df = 17$ ($P = 0.67$); $I^2 = 0\%$

Test for overall effect: $Z = 2.94$ ($P = 0.003$)

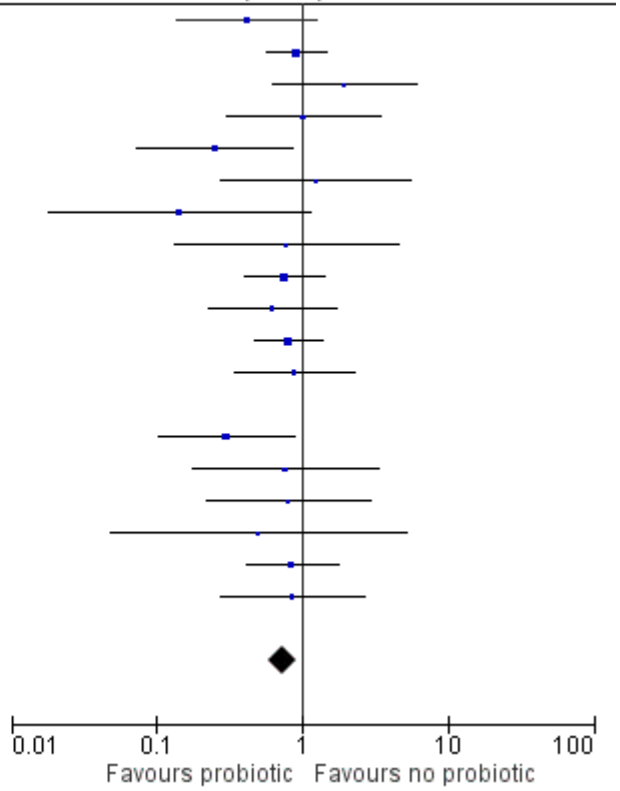

Figure 5

Forest plot: Effect of probiotics on time to full feeds (TFF)

Study or Subgroup	Probiotic Mean	Probiotic SD	Probiotic Total	No probiotic Mean	No probiotic SD	No probiotic Total	Weight	Mean Difference IV, Fixed, 95% CI
Braga 2010	15.2	5.2	119	17.4	5.7	112	8.0%	-2.20 [-3.61, -0.79]
Dashti 2014	13.83	10.99	69	16.11	14.82	67	0.8%	-2.28 [-6.68, 2.12]
Demirel 2013	11.7	4.74	135	13.2	12.67	136	3.1%	-1.50 [-3.77, 0.77]
Fernandez-Carrocera 2011	23	16.3	75	17.25	11.3	75	0.8%	5.75 [1.26, 10.24]
Oncel 2014	9.1	3.2	200	10.1	4.3	200	28.8%	-1.00 [-1.74, -0.26]
Roy 2014	11.22	5.04	56	15.41	8.07	56	2.6%	-4.19 [-6.68, -1.70]
Saengtawesin 2014	12	5.49	31	13.76	8.25	29	1.2%	-1.76 [-5.33, 1.81]
Samanta 2008	13.76	2.28	91	19.2	2.02	95	41.4%	-5.44 [-6.06, -4.82]
Sari 2011	17.3	8.7	110	18.3	9.8	111	2.7%	-1.00 [-3.44, 1.44]
Serce O 2013	11.9	7	104	12.6	7	104	4.4%	-0.70 [-2.60, 1.20]
Shadkam 2015	12.83	4.26	29	16.75	6.59	28	1.9%	-3.92 [-6.81, -1.03]
van Niekerk 2015	12.03	5.49	31	13.76	8.25	29	1.2%	-1.73 [-5.30, 1.84]
Yang 2011	14.7	5	31	17.1	4.2	31	3.0%	-2.40 [-4.70, -0.10]
Total (95% CI)			**1081**			**1073**	**100.0%**	**-3.09 [-3.49, -2.69]**

Heterogeneity: Chi² = 115.40, df = 12 (P < 0.00001); I² = 90%

Test for overall effect: Z = 15.19 (P < 0.00001)

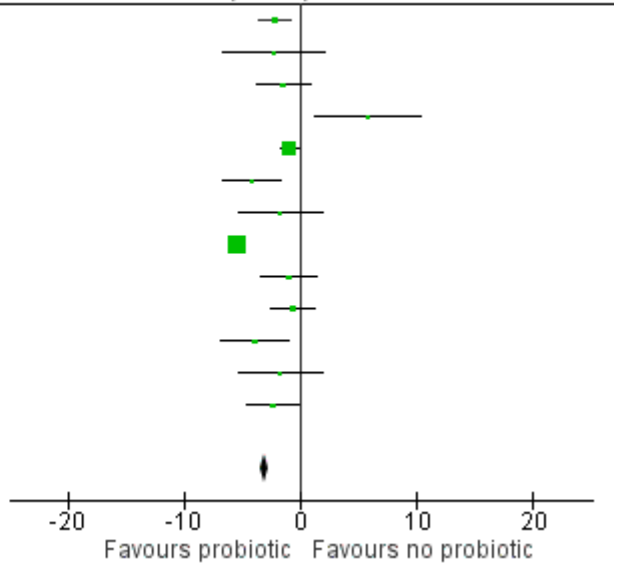

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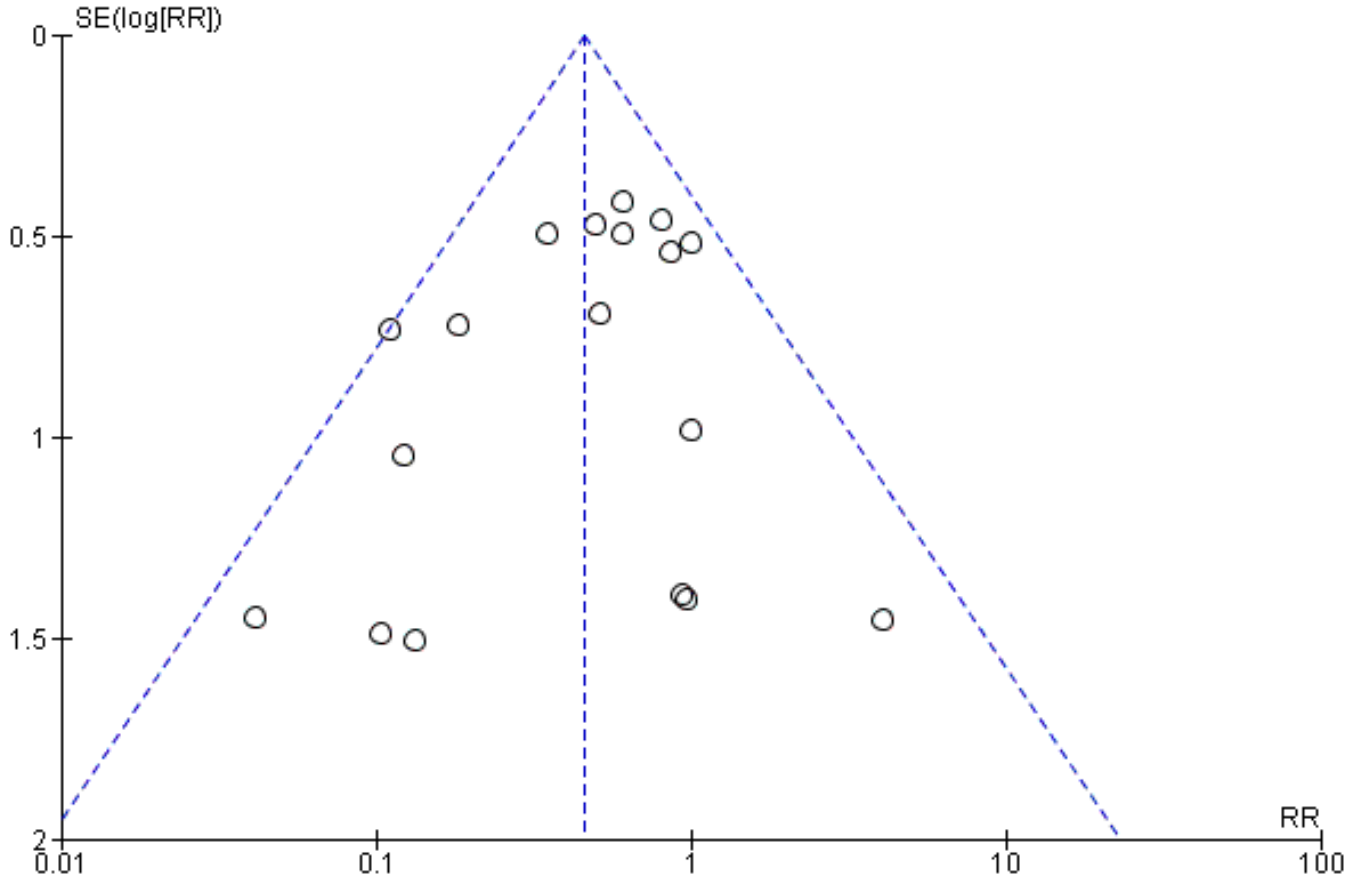
Figure 6
Funnel plot assessing publication bias



Appendix 1: Search strategy

When searched: December 2016 and January 2017

PubMed:

- ((("Infant, Newborn"[Mesh]) OR ("Infant, Extremely Premature"[Mesh] OR "Infant, Premature"[Mesh])) OR ("Infant, Low Birth Weight"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh])) AND "Probiotics"[Majr]: **716**
- (("Infant, Extremely Premature"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh] OR "Infant, Small for Gestational Age"[Mesh] OR "Infant, Premature, Diseases"[Mesh] OR "Infant, Premature"[Mesh] OR "Infant, Newborn, Diseases"[Mesh] OR "Infant, Newborn"[Mesh] OR "Infant, Low Birth Weight"[Mesh])) AND (((("Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Saccharomyces"[Mesh]): **774**
- probiotics and preterm infants: **350**
- probiotics and low birth weight infants: **146**
- probiotics and sepsis:**321**
- probiotics and ELGAN(extremely low gestational age) infants: **7**
- probiotics and Necrotizing enterocolitis: **381**

EMBASE: (probiotics.mp. or probiotic agent)/AND **(**preterm infant.mp. OR prematurity/low birth weight infant.mp. OR low birth weight/ very low birth weight infant.mp. OR very low birth weight/extremely low birth weight infant.mp. OR extremely low birth weight/small for gestational age.mp. OR small for date infant OR ELGAN.mp OR extremely low gestational age neonate.mp): **711**

CINAHL: 113

Cochrane: 84 trials

who.int /ictrp (WHO International Clinical Trials Registry Platform): 26, Relevant: 17, Recruiting: 4 (2 of the relevant)

PAS 2000-2014: 187 (probiotics), 68 (Bifidobacteria), 137 (Lactobacillus/ Lactobacilli), Saccharomyces (15)

PAS 2015: 17 (probiotics), 6 (Bifidobacteria), 4 (Lactobacillus/ Lactobacilli), Saccharomyces (2)

Grey literature search: Using term “probiotics and preterm infants”

- **Ntis.gov/:** 42, Relevant: 0; **Opengrey.eu/:** 2, Relevant: 0; **Trove.nla.gov.au**: 495, Duplicates:253, Not relevant:242

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5,6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6,7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6,7 and appendix1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Figure 1
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7,8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7.8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2 for each meta-analysis.	8

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2-5
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9,10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 3,4
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11,12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11,12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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Benefits of probiotics in preterm neonates in low and medium income countries - a systematic review of randomised controlled trials

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Benefits of probiotics in preterm neonates in low and medium income countries - a systematic review of randomised controlled trials

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Abstract

Objective: Although there is an overall reduction in under five mortality rate, the progress in reducing neonatal mortality rate has been very slow. Over the last 20 years, preterm births have steadily increased in low and medium income countries (LMIC) particularly in sub-Saharan Africa and South Asia. Preterm birth is associated with increased mortality and morbidity, particularly in LMICs. Based on systematic reviews of randomised controlled trials (RCT), many neonatal units in high income countries have adapted probiotics as standard of care for preterm neonates. Given their simplicity and affordability, we aimed to systematically review the safety and efficacy of probiotics in reducing mortality and morbidity in preterm neonates in LMICs.

Design: Systematic review and meta-analysis of randomised controlled trial

Data sources: Medline, EMBASE, Cochrane Central Register of Controlled Trials, CINAHL (Cumulative Index of Nursing and Allied Health Literature), and E-abstracts from the Pediatric Academic Society meetings and other paediatric and neonatal conference proceedings were searched in January 2017

Eligibility criteria: RCTs comparing probiotics vs. placebo/no probiotic in preterm neonates (gestation <37 weeks) conducted in LMICs.

Results: Total 23 (N=4783) RCTs from 4 continents and 10 LMICs, were eligible for inclusion in the meta-analysis using fixed effects model. The risk of NEC [RR: 0·46(95% CI: 0·34, 0·61) p <0.00001, NNT: 25 [95% CI: 20, 50], late onset sepsis (LOS) [RR: 0·80(95% CI: 0·71, 0·91) p=0.0009, NNT: 25 [95% CI: 17, 100] and death [RR: 0·73(95% CI: 0·59, 0·90) p=0.003, NNT: 50 [95% CI: 25, 100] was significantly lower. The results were significant on random effects model analysis and after excluding studies with high risk of bias. No significant adverse effects were reported.

Conclusion: Probiotics have significant potential to reduce mortality and morbidity (e.g. NEC, LOS) in preterm infants in LMICs

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Strengths and limitations of this study

- To our knowledge this is the first systematic review focusing on RCTs of probiotics in preterm neonates in LMICs.
- Beneficial effects of probiotics in reducing sepsis, NEC and mortality are significant considering the United Nation's MDG4 and UN Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying Every Woman, Every Child initiative, Every Newborn Action plan (ENAP), and the burden of prematurity in LMICs
- The limitations include variations in the probiotic protocols in the included RCTs. Furthermore nearly 40% of the included trials carried a high risk of bias in many domains of assessment.

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Introduction

The UNICEF 2010 report showed that the global burden of under five mortality was reduced by one third compared to 1990s; however progress in reducing neonatal mortality has been slow. [1-3] Almost 40% of under five deaths occur during the neonatal period and majority of these deaths occur in Sub-Saharan Africa, South Asia, and Oceania. An estimated 98% of all neonatal deaths occur in low and medium income countries (LMIC). [4-6] Out of 135 million births each year, 3.1 million have died within the neonatal period and nearly 35% of these deaths occur in preterm neonates.[2,5] It may be perceived that prematurity is not a problem of LMICs. However, it is important to note that only 8.6% of preterm births occur in developed countries[5] Over the last 20 years, the number of preterm births has steadily increased to 9.1million as of 2010 in the regions of sub-Saharan Africa and South Asia. Preterm birth is associated with increased risk of mortality, and morbidity including late onset sepsis (LOS), necrotising enterocolitis (NEC), feeding difficulties, and long term neurodevelopmental impairment (NDI). [6-8] Although survival of preterm neonates has improved in some LMICs, morbidities such as NEC and LOS are still a major issue.[5,9-12] Considering the United Nation's millennium developmental goal (MDG-4), and the United Nation Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying 'Every Woman, Every Child initiative, Every Newborn Action plan' (ENAP), it is important to develop cost-effective simple strategies to reduce the mortality and morbidity associated with prematurity in LMICs.[13]

The World Health Organisation (WHO) defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".[14] Probiotics have been shown to significantly reduce the risk of NEC, all-cause mortality, LOS and facilitate feed tolerance in preterm very low birth weight (VLBW) neonates.[15-17] The mechanisms of benefits of probiotics include gut barrier enhancement, immune response

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modulation (e.g. TLR4 receptor, nuclear factor-B, inflammatory cytokines), and direct inhibition of gut colonisation by pathogens.[18-22] Many developed countries are already using probiotics routinely in preterm neonates for prevention of NEC.[23-32] It has been suggested that probiotics may have a role in LMICs for prevention, treatment of acute gastrointestinal diseases, particularly in children with HIV infection.[33-36] Given their simplicity and affordability, we aimed to systematically review the safety and efficacy of probiotics in reducing the risk of mortality and morbidity in preterm neonates in LMICs.

METHODS

Guidelines from the Cochrane Neonatal Review Group (http://neonatal.cochrane.org/resources-review-authors),[37] Centre for Reviews and Dissemination (http://www.york.ac.uk/crd/guidance/),[38] and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [39] were followed for undertaking and reporting this systematic review and meta-analysis. Ethics approval was not required.

Eligibility Criteria:

Types of studies: Only randomised controlled trials (RCTs) were included in the review. Observational studies, narrative/systematic reviews, case reports, letters, editorials and commentaries were excluded, but read to identify potential additional studies.

Types of participants: Preterm neonates born at a gestational age (GA) <37 weeks or low birth weight (LBW: <2500 grams) or both (Same criteria as the Cochrane review, 2014).[15]

Setting: Only RCTs from LMICs were included. LMICs were defined as per the World Bank guidelines which include countries with gross national income (GNI) per capita of under $12736/year.[40]

Intervention and comparison: Enteral administration of probiotic supplement versus control (placebo/no probiotic).

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Outcomes: All-cause mortality, LOS (Positive blood/CSF culture on a sample collected 48-72 hours after birth), Definite NEC (Stage ≥II modified Bell staging)[41] and time to full enteral feeds. (TFF: 120ml/kg/day).

Search strategy: The databases Medline searched via PubMed (www-ncbi-nlm-nih-gov, 1966-2017), EMBASE (Excerpta Medica dataBASE) via Ovid (http://ovidsp.tx.ovid.com, 1980-2017), Cochrane Central Register of Controlled Trials (www.thecochranelibrary.com, through January 2017), CINAHL (Cumulative Index of Nursing and Allied Health Literature) via OVID (http://ovidsp.tx.ovid.com, 1980- January 2017), and E-abstracts from the Pediatric Academic Society meetings (www.abstracts2view.com/pasall, 2000- January 2017) were searched in January 2017. Abstracts of other conference proceedings such as European Academy of Paediatric Societies (EAPS) and the British Maternal and Fetal Medicine Society were searched in EMBASE. 'Google Scholar' was searched for articles that might not have been cited in the standard medical databases. Grey literature was searched using the national technical information services (http://www.ntis.gov/), Open Grey (http://www.opengrey.eu/), and Trove (http://trove.nla.gov.au/). We have also searched LILACS and Caribmed via the BIREME/PAHO/WHO - Latin American and Caribbean Center on Health Sciences Information (http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/) using broad terminologies Probiotics OR Probiotic Or Bifidobacterium OR Bifidobacteria OR Lactobacillus OR Lactobacilli OR Saccharomyces. We also searched https://clinicaltrials.gov, http://www.who.int/ictrp/en/, and www.bioportfolio.com for ongoing RCTs. The reference lists of eligible studies and review articles were searched to identify additional studies. Reviewers SR, GJ and GD conducted the literature search independently. No language restriction was applied. The non-English studies were identified by reading the recent systematic reviews of probiotic supplementation for reducing the risk of NEC,[42,43] and from cross references of individual studies. Full texts of all non-English

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studies were obtained via University of Sydney and Department of New South Wales (NSW) health library. A research officer from the NSW Health, University of Sydney translated the articles. Attempts were made to contact the authors for additional data and clarification of methods. Only published data were used for those studies, where available.

PubMed was searched using the following terminology: ((("Infant, Newborn"[Mesh]) OR ("Infant, Extremely Premature"[Mesh] OR "Infant, Premature"[Mesh])) OR ("Infant, Low Birth Weight"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh])) AND "Probiotics"[Majr]. It was also searched using (("Infant, Extremely Premature"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh] OR "Infant, Small for Gestational Age"[Mesh] OR "Infant, Premature, Diseases"[Mesh] OR "Infant, Premature"[Mesh] OR "Infant, Newborn, Diseases"[Mesh] OR "Infant, Newborn"[Mesh] OR "Infant, Low Birth Weight"[Mesh])) AND ((("Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Saccharomyces"[Mesh]). The other databases were searched using similar terminologies. The detailed search terminology is given in appendix 1.

Study selection: The abstracts of citations obtained from the initial broad search were read independently by reviewers SR, GJ, and GD, to identify potentially eligible studies. Full-text articles of these studies were obtained and assessed for eligibility by reviewers SR, GJ and GD independently, using the predefined eligibility criteria. Differences in opinion were resolved by group discussion to reach consensus. Care was taken to ensure that multiple publications of the same study were excluded to avoid data duplication.

Data extraction: Reviewers GD, SR and GJ extracted the data independently using a data collection form designed for this review. Information about the study design and outcomes was verified by all reviewers. Discrepancies during the data extraction process were resolved by group discussion. We contacted authors for additional information/clarifications.

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Assessment of risk of bias (ROB): ROB was assessed using the Cochrane "Risk of Bias Assessment Tool".[44] Authors GD, SR and GJ independently assessed the ROB in all domains including random number generation, allocation concealment, blinding of intervention and outcome assessors, completeness of follow up, selectivity of reporting and other potential sources of bias. For each domain, the ROB was assessed as low, high or unclear risk based on the Cochrane Collaboration guidelines.

Data synthesis: Meta-analysis was conducted using Review Manager 5•3 (Cochrane Collaboration, Nordic Cochrane Centre). Fixed-effects model (FEM) (Mantel–Haenszel method) was used. Random-effects model (REM) analysis was conducted to ensure that the results and conclusions were not influenced by the type of model used for the meta-analysis. Effect size was expressed as risk ratio (RR) and 95% % confidence interval (CI).

Statistical heterogeneity was assessed by the $\chi 2$ test, I2 statistic and visual inspection of the forest plot (overlap of CIs). A p value <0.1 on $\chi 2$ statistic was considered to indicate heterogeneity. An I^2 statistic values were interpreted as per the Cochrane handbook guidelines as follows: 0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity.[37] The risk of publication bias was assessed by visual inspection of the funnel plot.[45]

Subgroup analysis: a) Low ROB: random sequence generation and allocation concealment b) Premature neonates less than 34 weeks gestation or birth weight less than 1500g.; c) Where *Bifidobacterium* was part of the supplementation; d) Where *Lactobacillus* was part of the supplementation; e). Single strain probiotic were used and f) Multiple strain probiotics were used.

Summary of findings table: The key information concerning the quality of evidence, the magnitude of effect of the intervention and the sum of available data on the main outcome

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was presented in the 'summary of findings table' as per the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) guidelines.[44]

Funding source: Not required

Results: The literature search retrieved 1926 potential relevant citations. After carefully reviewing the abstracts, 1814 studies were excluded: Reviews: 378, observational studies: 187, commentaries: 49, case reports: 147, RCTs in adult and paediatric population: 53, and non-relevant studies: 982. Finally 23 RCTs (n=4783) conducted in 10 different LMICs in 4 continents were included in the meta-analysis. [12,46-67] The search strategy results are given in appendix 1. The flow diagram of study selection process is given in **figure 1**. The characteristics of the included studies are given in **table 1**. Out of the 23 included studies, Single-strain probiotics were used in 11 studies, whereas 12 used multiple strains. *Lactobacillus* was part of the supplementation in 13 studies; *Bifidobacterium* was part of the supplementation in 11 studies and saccharomyces in 3 studies. **(Table 1)**

ROB of included studies: A total of 14/23 (60%) included studies were judged to have low ROB for the domain of 'random sequence generation', and (56%) were considered to have low ROB for 'allocation concealment'. **(Table 2)**

Effect of probiotics on ≥ Stage II (definite) NEC (Figure 2): Data on definite NEC was reported by 20 trials (N=4022).[12,46-53,55,56,58-65,67] A higher proportion of neonates in the control group developed definite NEC compared with the probiotic group [65/2065 (3.1%) vs. 135/1957 (6.9%)]. Meta-analysis using a FEM estimated a lower risk [RR: 0.46 (95% CI: 0.34, 0.61), p<0.00001] of NEC in the probiotic group. There was no significant heterogeneity (I^2 =19%, p=0·22) among the trials. The numbers needed to treat (NNT) with probiotics to prevent one case of NEC was 25 [95% CI: 20, 50].

Effect of probiotics on LOS (Figure 3): Data from 18 trials[12,46,47,49,51-54,56-62,64,65,67] (N=4062) showed that a higher proportion of neonates in the control group developed LOS

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compared with those in the probiotic group [308/2076 (14.5%) vs. 358/1986 (18%)]. Meta-analysis using a FEM estimated a lower risk [RR: 0.80 (95% CI: 0.71, 0.91), p=0.0009] of LOS in the probiotic group. There was no significant heterogeneity (I^2 =25%, p=0.16) among the trials. The NNT with probiotics to prevent one case of LOS was 25 (95% CI: 17, 50).

Effect of probiotics on all cause mortality (Figure 4): Data from 19 trials (N=4196),[12,46-49 51-54 56-65] showed reduced risk of death due to all causes in the probiotic vs. control group [137/2148 (6.37%) vs. 176/2048 (8.59%)] Meta-analysis using a FEM estimated a lower risk [RR: 0.73(95% CI: 0.59, 0.90), p=0.003] of death in the probiotic group. No significant heterogeneity was noted between the trials (I^2 =0%, p=0.67). The NNT to prevent one death by probiotic supplement was 50 (95% CI: 25, 100).

Effect of probiotics on TFF (Figure 5) Meta-analysis of data (N=2154) from 13 trials [12,47-49, 53,56,59-63,65,66] showed significant reduction in TFF in the probiotics vs. control group [MD=-3.09 days (95% CI: -3.49, -2.69), p<0.00001]. However, there was significant heterogeneity (I^2= 90%, p< 0.00001) among the trials. These results were hence checked by using REM and remained significant [MD=-1.95 days (95% CI: -3.44, -0.45), p=0.01].

Subgroup analysis: The beneficial effects continued to be observed in studies a) Low ROB: random sequence generation and allocation concealment **(Table 3)** b). That only included infants with gestational age <34 weeks or birth weight <1500g; c) Where *Bifidobacterium* was part of the supplementation; d) Where *Lactobacillus* was part of the supplementation; e). Single strain probiotics were used and f) Multiple strain supplements were used; however, on REM meta-analysis, statistical significance was lost for some of these analyses **(Table 4).** The overall evidence according to GRADE guidelines is provided as a summary of findings table **(Table 5)**. The evidence was deemed high in view of the large sample size, low risk of bias in majority (14/20) of the included studies, narrow confidence intervals around the effect

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size estimate, very low p value for effect size estimate and mild statistical heterogeneity. Visual inspection of the funnel plot suggested that there was no publication bias **(Figure 6).**

Safety: None of the studies reported any significant adverse effects including probiotic sepsis.

Discussion:

The results of our systematic review of 23 RCTs (N=4783) conducted in ten LMICs across four continents show that probiotic supplementation in preterm neonates (born <37 weeks) significantly reduces the risk of all-cause mortality, LOS and NEC in such a set up. The limitations of this review include variations in types of probiotics used in different studies and limitations of study qualities in few studies. The strengths of our review include reliable results, considering the robust methodology, number of trials from different LMICs, large sample size, low/no statistical heterogeneity, and the small p values indicating the low probability of chance. Summary findings as per GRADE guidelines confirm the high quality evidence (Table 5). To our knowledge this is the first systematic review focusing on RCTs of probiotics in preterm neonates in LMICs. Our results are significant considering the United Nation's MDG4 and UN Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying Every Woman, Every Child initiative, Every Newborn Action plan (ENAP), and the burden of prematurity in LMICs.[4,5,13]

The incidence of prematurity is significantly increasing in LMICs compared to Europe or North America. There are issues related to reporting of preterm births and outcomes in LMICs.[68] However the studies funded by the WHO estimate 13 million preterm births/year in LMICs with 11 million (85%) of these being concentrated in Africa and Asia, ~0.5 million each in Europe and North America (excluding Mexico) and 0.9 million in Latin America and the Caribbean.[69] The highest rates (11.9%) and number (seven million) of preterm births were in Africa, and Asia respectively. Mortality and morbidities such as LOS, NEC and feeding

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difficulties are major issues in preterm neonates. Although specific data from LMICs is not available, ~one million preterm neonates die every year, predominantly due to sepsis, and long-term impairment in survivors is becoming an important issue. [70]

Consistent with our recent systematic review[71], our results show that probiotics reduced the risk of not only NEC and all-cause mortality but also of LOS in preterm neonates. [RR: 0.81 (95% CI: 0.71, 0.92), p=0.001]. The reduction of LOS by probiotics is important considering that neonatal sepsis is responsible for nearly a 3rd all neonatal deaths in LMICs.[19, 20,22,72-77]

It is important to note that the burden of NEC is as significant in LMICs as in high income countries. The incidence and severity of NEC is higher in LMICs and includes up to 15% cases of NEC totalis with ~100% mortality.[9,12] It occurs not only in VLBW and ELBW neonates but also in preterm neonates with higher birth weight. Lack of antenatal steroids and being small for gestational age (SGA) due to intrauterine growth restriction (IUGR) are known risk factors for NEC.[78] The reason for higher incidence of NEC in LMICs could include the higher numbers of preterm 'SGA-IUGR' births and limited coverage of antenatal steroids.[79,80] The NEC related mortality and morbidity is almost entirely due to progression of the illness from stage II to stage III. Management of surgical NEC is difficult in LMIC considering the limited resources. Primary prevention of NEC is therefore an important strategy for reducing the health burden of the condition in LMICs. Considering the effect size with regards to reduced risk of NEC, the benefits of probiotics in LMIC could not be overemphasised.

The issue of implementing probiotics for preterm neonates in LMICs is complex. The options include either reconfirming their safety and efficacy in large definitive RCTs in LMICs or adopting their routine use based on current evidence. Conducting large multicentre trials and accessing proven safe and effective probiotics is difficult, especially in resource limited set ups.[34] Apart from the significant budget the difficulties include regulatory hurdles, and

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logistics of importing a probiotic product, maintaining cold chain, and providing ongoing independent safety and quality control. However there are recent examples of large RCTs conducted successfully in community settings in LMICs.[81,82] Neonatal demographic characteristics such as gestation and IUGR, are an important issue in conducting RCTs in LMICs as they determine the risk of NEC, duration of probiotic supplementation, and the cost-benefit ratio. It is also important to note that many RCTs have used different probiotic/s and probiotic activity could be strain specific.

Knowledge of the pattern of gut colonisation in preterm neonates in a given set up is important before using probiotics for research or routine use. Dutta et al have reported abnormal intestinal colonization patterns in the first week of life in VLBW neonates in their level III neonatal intensive care unit in India.[52] On day one, 45% neonates had sterile guts, and by day three, all were colonized predominantly by E. coli, K. pneumoniae and Enterococcus fecalis. Only one isolate had lactobacilli and bifidobacteria were not detected during the study period. Formula feeding was associated with E. coli colonization. Results of completed [82] and ongoing trials such as NCT02552706 will be important.[83]

Probiotic sepsis, antibiotic resistance, and altered immune responses in the long run, are the potential adverse effects of probiotics in preterm neonates. Availability of killed or inactivated probiotic strains with clinically proven benefits may help not only in avoiding such adverse effects but also in avoiding the need to maintain the cold chain. Awad et al have compared the effect of oral killed (KP) versus living *lactobacillus acidophilus* (LP) in reducing the incidence of LOS and NEC in neonates.[46] Both LP and KP reduced the risk of NEC [Absolute risk reduction (ARR): 16%, 15%, respectively] and LOS (ARR: 18%) significantly compared with placebo. LOS and NEC was reduced significantly in neonates colonised versus not colonised by *lactobacillus* at day 7 (27.9 vs. 85.9%, 0 vs. 7.8%) and day 14 (48.7 vs. 91.7% for LOS and 0 vs. 20.8% for NEC). KP retained the benefits similar to LP

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on comparison between all groups. Given the global implications of these results, the benefits of inactivated/killed probiotics need to be assessed in further large definitive trials.

In summary our results indicate that probiotics are effective in significantly reducing the risk of all-cause mortality, LOS, and NEC in preterm VLBW neonates in LMICs. Considering the burden of death, disease (NEC, LOS), and suboptimal nutrition in preterm neonates in LMICs, cooperation between various stake holders (e.g. industry, scientists, regulatory agencies) is warranted to either develop or to improve access to high quality safe and effective probiotics in such set ups. Support from organisations such as the WHO is important in providing access to probiotics for the countries (e.g. sub-Saharan Africa) where most prematurity related deaths occur. Whether probiotics could be used for research and/or routine use in preterm neonates in LMICs will depend on the national health priorities, resources, and ethics.

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Authors' contributions: Dr Deshpande conceptualized and designed the study, performed an independent literature search, selected studies for inclusion, extracted and interpreted data, assessed risk of bias of included studies, handled the meta-analysis software, oversaw translation of manuscripts in the Chinese language and wrote the first and final drafts of the manuscript; Dr Athalye-Jape performed an independent literature search, selected studies for inclusion, contacted authors for additional information where necessary, extracted and interpreted the data, checked the data entered by Dr Deshpande on the meta-analysis software, assessed the risk of bias of included studies, and helped with the first and the final draft of the manuscript; Dr Rao performed an independent literature search, selected studies

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for inclusion, verified the extracted data, assessed risk of bias, interpreted data, and helped with the first and the final draft of the manuscript; Prof Patole supervised the project, acted as referee author in case of differences of opinion between the first 3 authors, interpreted the data, and supervised the first and approved the final versions of the manuscript; and all authors approved the final manuscript as submitted.

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Table 1: **Characteristics of included studies**

Study ID/	Location	Study characteristics
1. Awad 2010[40]	**Egypt**	**Participants:** All neonates admitted to nursery, 28-41 weeks and weight 1.1-4.3 kg **Intervention and dose:** Killed probiotic/KP (*L. acidophilus,* 6× 10^9 CFU) vs. Living probiotic/LP (*L. acidophilus,* 6× 10^9 CFU) vs. Placebo **Duration of supplementation:** commenced on D1, duration NA **N=150** (60 vs. 60 vs.30), **Preterm: 89** (37 vs. 36 vs. 16) **Type of milk:** details NA **Type of delivery:** Preterm CS: KP (57%) vs. LP (56%) vs. Placebo (75%) **Primary outcome: All outcomes for LP vs. KP vs. Controls:** Incidence of neonatal sepsis (18/36, 50% vs. 25/37,68% vs. 12/16, 75%; p=0.251) and NEC (0/36 vs. 1/37 vs.5/16; p=0.000) neonates and evaluation of efficacy of a KP **Other outcome: Mortality:** 4/36 (11.1%) vs. 12/37(32.4%) vs. 5/16(31.3%) p=0.076
2. Braga 2010[41]	**Brazil**	**Participants:** Preterm infants 750-1499g **Intervention and dose:** (*L. casei* + *B. breve* : 3.5x10^7 to 3.5x10^9 CFU) vs. No probiotic **Duration of supplementation::** Once daily from the second day of life until day 30 **N =231** (Probiotics: 119, Controls: 112) **Type of milk**: EBM/ PDHM **Type of delivery**: CS 53.8% vs. 49.1 % **Primary outcome:** ≥ Stage II NEC (0/119, 0% vs. 4/112, 3.6%) **Other outcomes: LOS:** 40/119 (33.6% vs 42/112 (37.5%)**, Mortality:** 26/119 (21.8%) vs. 27/112(24.1%)
3. Dashti 2014[42]	**Iran**	**Participants:** Preterm infants 700-1800 g **Intervention and dose:** (*L. acidophilus, L. rhamnosus, B. longum, L. bulgaricus, L. casei, S. thermophilus, B. breve,* and *Bifidobacterium*: total 1x10^9 CFU/sachet) vs. placebo powder **Duration of supplementation::** Once daily from first feed of life until discharge **N =136** (Probiotics: 69, Controls:67) **Type of milk**: EBM/ formula milk **Type of delivery**: CS 82.4% vs. 17.6 % **Primary outcome:** ≥ Stage II NEC (2/69, 2.9% vs. 1/67, 1.5%) **Other outcomes: Mortality:** 8/69 (11.6%) vs. 4/67(5.97%)
4. Demirel, Erdeve 2013[43]	**Turkey**	**Participants:** Preterm infants ≤32 w and ≤1500g **Intervention and dose: :** S. *boulardii,* 5x10^9CFU vs. no probiotic **Duration of supplementation:** NA **N=271** (Probiotic: 135, Controls: 136) **Type of milk**: EBM/ Formula **Type of delivery**: CS 77.7% vs. 83% **Primary outcome:** NEC ≥ stage 2(6/135, 4.4% vs. 7/136, 5.1%) p=1, **Mortality:**(5/135,3.7% vs. 5/136, 3.7%) p=1 **Other outcomes: LOS:** 20/135 (14.9%) vs 21/136 (15.4%) p=0.906**, Feed intolerance:**30/135(22.2%) vs. 62/136(46%), p<0.001
5. Deng 2010[44]	**China**	**Participants:** 125 preterm infants, <37 weeks, <2500 g at birth **Intervention and dose:** *B. longum, L. acidophilus, Enterococcus fecalis*, triple viable powder oral or nasal Bifico plus powder / capsules. For birth weight <1500 g: 0.33× 10^7 CFU of each probiotic twice daily and >1500 g: 0.5× 10^7 of each probiotic twice daily, Control: sterile warm water **Duration of supplementation:** commenced from first feed till 14 days of life

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		N=125 (**62** Controls 33.2 ± 2.3 weeks vs **63** Probiotic group 32.4 ± 2.8 weeks), **Type of Milk:** EBM/preterm formula **Type of Delivery:** NA **Primary outcome: NEC: Controls**: Bell Stage I (1/62, 1.6%), Bell Stage II (4/62, 6.5%), Bell Stage III (4/62, 6.5%) vs T**reatment** Bell Stage I (1/63,1.6%), Bell Stage II (1/63, 1.6%) **Other outcomes: LOS, Mortality:** NA
6. Dilli 2015[45]	**Turkey**	**Participants:** VLBW infants with a gestation of <32w and birth weight<1500g **Intervention and dose:** *B. lactis* (5x10^9 CFU) vs. Placebo (maltodextrin) **Duration of supplementation:** From day 8 of life, once daily until discharge or a maximum of 8 weeks **N=200** (Probiotic 100, Placebo: 100) **Type of milk:** EBM/Formula **Type of delivery: CS**: 35/100 (35%) vs. 37/100 (37%) **Primary outcome: NEC (≥stage 2):**2/100(2%) vs.18/100 (18%), p<0.001 **Other outcomes: LOS:** 8/100 (8%) vs 13/100 (13%), p=0.6, Mortality: 3/100 (3%) vs. 12/100 (12%), p=0.003, Time to full enteral feeds^ (150ml/kg/day): 18(14-23) days vs. 25(15-37) days, p<0.001
7. Dutta 2015[46]	**India**	**Participants:** Preterm infants 27-33 weeks gestation **Intervention:** High dose (10 billion CFU: *L. acidophilus, L. rhamnosus, B. longum, S. boulardii*) **vs.** Low dose (1 billion CFU*: L. acidophilus, L. rhamnosus, B. longum, S. boulardii*) vs. Placebo (potato starch, maltodextrin) **Duration of supplementation: Probiotic groups: (A)**: High dose for 21 days, (C): Low dose for 21 days, (B): High dose short course (D1-D14 and D15-D21) **N: Probiotic (114)** vs. **Placebo (35)** **Type of milk:** EBM /formula **Type of delivery:** Probiotic group vs. Placebo: SVD (69% vs. 60%), CS: data NA **Primary outcome: Stool colonisation rates on D14, D21, D28 with 3 different probiotic regimens** (*Lactobacillus* and *Bifidobacterium* colonisation was significantly higher in groups A, B, and C vs. placebo respectively. Groups A, B, and C did not differ from each other. There were trends toward more CFU of *Lactobacillus* and *Bifidobacterium* per ml of stool in group A vs. B and B vs. C. Groups A and B and spontaneous preterm labor (SPL) independently predicted high Lactobacillus counts on day 28; groups A, B, and C and SPL predicted high *Bifidobacterium* counts) **Other outcomes: LOS:** 10/114 (8.8%) vs. 6/35 (17.1%), p=0.14, **Mortality**:8/114(7%) vs.2/35(12.7%), p=0.85, **NEC (≥stage 2):** 6/114(5.3%) vs 0/35(0%), p=0.35
8. Fernandez-Carrocera 2013[47]	**Mexico**	**Participants:** Preterm infants<1500g **Intervention and dosage:** Multispecies probiotic product (*L. acidophilus +L. rhamnosus +L. casei+ L. plantarum+ B. infantis+ S. thermophilus)* vs. No probiotic **Duration of supplementation:** From the day of commencement of enteral feeds, once daily. Actual Duration: NA **N=150** (Probiotics:75, Controls: 75) **Type of milk:** EBM/ Formula **Type of delivery:** data not available **Primary outcome:**≥ Stage 2 NEC: 6/75(8%) vs 12/75(16%), p=0.142 **Other outcomes: LOS:** 42/75 (56%) vs 44/75 (58.7%), p=NA, **Mortality:** 1/75(1.3%) vs. 7/75(9.3%), p=0.063
9. Hua 2014[48]	**China**	**Participants:** Preterm infants<37 weeks **Intervention and dosage:** Probiotic Jin Shuang QI (*L. acidophilus, S. thermophilus, Bifidobacterium*) 5 x 10^7 CFU/day. Vs no probiotic **Duration of supplementation:** From the day of commencement of enteral feeds, once daily. Duration of Supplementation: not clear **N=257** (Probiotics:119, Controls: 138)

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		Type of milk: EBM/ Formula **Type of delivery**: CS 55.5% vs 64.5% **Primary outcome: Stool colonisation by drug resistant bacteria** (No difference in both groups, p>0.05) **Other outcome: LOS:** 2/119(1.7%) vs. 8/138 (5.8%); p-0.168, **NEC (stage NS):** 0/119 vs. 2/138; p=0.501,**Mortality:** 2/119 vs. 2/138
10. Huang 2009[49]	**China**	**Participants:** Preterm infants 28-32 weeks and <1500g **Intervention and dosage:** *Bifidobacterium* (50 million live bacteria/capsule) $0.25x10^8$ live bacteria oral/nasally twice daily vs. non-treatment (control) **Duration of supplementation:** From 7 days till 14 days of age **N=183** (Probiotic: 95, Control: 88) **Type of milk:** Not stated **Type of Delivery:** NA **Primary outcomes: NEC:** 2/95 (2.1%), both Bell's stage 1 vs. 9/88 (10.23%): Bell's stage 1:6, stage 2:2, stage 3:1 (p<0.01), Body mass changes/**Weight gain*:** Probiotic group: 8.109 ± 2. 127 g vs. Control group 6. 489 ± 2. 327 g (p<0.01) **Other outcomes: LOS, Death, TFF:** NA**, gut colonisation:** Post 7d of treatment, the two groups' intestinal bacteria and bacteria ratio of the total number of cocci and rods, the differences were statistically significant (P <0. 01). Rod bacteria ratio before and after preventive treatment groups showed no significant difference (P> 0 05.); in the control group rod bacteria ratio difference was statistically significant (P <0. 01)
11. Oncel 2014[50]	**Turkey**	**Participants:** Preterm Infants≤32w and <1500g **Intervention and dosage:** *L. reuteri DSM 17938* in oil based suspension,$1x10^8$ CFU/day vs Placebo (Oil based suspension without probiotics) **Duration of supplementation:** From the time of first enteral feeds until discharge **N=400** (Probiotics: 200, Placebo: 200) **Type of milk:** EBM/Preterm formula, **Type of delivery**: CS 75% vs. 76% **Primary outcome: Probiotics vs. Controls: ≥ Stage 2 NEC or death:** 20/200(10%) vs. 27/200(13.5%);p=0.27, **NEC (≥stage 2):**8/200(4%) vs.10/200(5%);p=0.63 **Other outcomes: Late Onset Sepsis:** 13/200 (6.5%) vs 25/200 (12.5%);p=0.041, **Time to full feeds*:**9.1±3.2 vs. 10.1±4.3 days; p=0.006, **Hospital stay^:**38(10-131) vs 46(10-180) days; p=0.022**, Feed intolerance:**56/200(28%) vs. 79/200(39.5%);p=0.015
12.Qiao 2012[51]	**China**	**Participants:** Preterm 28-34 weeks GA, >1000 g, <72 h life **Intervention:** *Bifidobacterium, Lactobacillus, Streptococcus thermophiles*, 0.5g per bag **Duration of supplementation:** 0.5 bag three times daily for 3 days after admission to hospital **N=287** (Probiotic: 149 vs Control 138) **Type of milk:** Not stated **Type of Delivery:** No Stats on CS/type of delivery **Primary outcomes:** Time to full oral feeds (7.3 d vs 16.9 d); p<0.05, time to full enteral nutrition (9.8 d vs 16.9 d); p<0.05, LOS (6.7% vs 15.2%); p<0.05, NEC (3.4% vs 10.9%); p<0.05, hospitalisation time (25.0 d vs 30.8 d); p:NA, Mortality*: (6.0 ± 4.0)% and (9.0 ± 6. 5)%;p>0.05
13. Rojas 2012[52]	**Columbia**	**Participants:** Preterm Infants≤2000g **Intervention and dosage:** *L. reuteri DSM 17938*, $1x10^8$ CFU, once daily vs Placebo (Oil based suspension without probiotics) **Duration of supplementation:** Commenced within 48 hours of life. Duration: NA **N=750** (Probiotics:372, Placebo:378) **Type of milk:** EBM/Formula **Type of delivery**: VD non-instrumental: 16% (Study) vs. 17% (Placebo), VD instrumental: 0% (Study) vs. 0.5% (Placebo), Elective CS: 18% (Study) vs. 17% (Placebo), Non Elective CS 65% (Study) vs. 65% (Placebo)

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		Primary outcome: Nosocomial infection and mortality:57/372(15.3%) vs. 67/378(17.7%);p=0.38**, Death:** 22/372(5.9%) vs. 28/378(7.4%);p=0.41 **Other outcomes: LOS:** 24/372 (6.5%) vs 17/378 (4.5%);p=0.24, **Duration of hospitalisation^**: 20(11-33) vs. 20(11-38) days; p=0.53
14. Roy 2014[53]	**India**	**Participants:** Preterm infants<37w and birth weight<2500g **Intervention and dosage:** Half of the one gram sachet that contained *L. acidophilus* 1.25x10^9 + *B. longum* 0.125x10^9 + *B. bifidum* 0.125x10^9+ *B. lactis* 1x10^9 vs. Sterile water **Duration of supplementation**: Commenced within 72 hours of birth for six weeks or until discharge **N=112** (Probiotics: 56, Placebo: 56) **Type of milk**: EBM **Type of delivery**: CS 83.9% vs. 76.8% **Primary outcome**: **Enteric fungal colonisation*:** 3.03± 2.33 ×10^5 CFU vs. 3± 1.5×10^5; p=0.03 and **LOS (bacterial and fungal):** 31/56(55.4%) vs. 42/56(75%); p=0.02 **Other outcome: TFEF*:**11.22± 5.04 vs. 15.41± 8.07 days; p=0.016
15.Saengtawesin 2014[54]	**Thailand**	**Participants:** Preterm(<34 w) and VLBW (<1500g) infants **Intervention and dosage:** Probiotic mixture [*L. acidophilus* + *B. bifidum* each 1 x10^9 CFU/250mg], 125mg/kg twice daily vs. No probiotic **Duration of supplementation:** NA **N=60** (Probiotics: 31, Controls:29) **Type of milk:** EBM/preterm formula **Type of Delivery:** CS 67.7% vs. 62% **Primary outcome: NEC≥ stage 2:** 1 (3.2%) vs 1 (3.4%); p=0.74 **Other outcomes: LOS:** 2(6.45%) vs. 1(3.44%); p=0.53, **TFEF*:** 12.03 ± 5.49 days vs. 13.76 ± 8.25 days (p = 0.64).
16. Samanta 2009[12]	**India**	**Participants:** Preterm(<32 w) and VLBW (<1500g) infants **Intervention and dosage:** Probiotic mixture [*B. infantis* + *B. bifidum* + *B. longum* + *L. acidophilus*, each 2.5x10^9 CFU], administered twice daily vs. No probiotic **Duration of supplementation:** NA **N=186** (Probiotics: 91, Controls: 95) **Type of milk:** EBM **Type of Delivery:** CS 46.15% vs 49.47% **Primary outcomes: Incidence of NEC(≥stage 2):**5/91(1.1%) vs.15/95(15.8%);p=0.042**, Death due to NEC:** Overall death: 4/91(4.4%) vs. 14/95(14.7%); p=0.032**, Feed tolerance: Time to full feeds*:** 13.76 ± 2.28 vs. 19.2 ± 2.02 days; p<0.001 **Other outcomes: LOS:** 13/91 (14.3%) vs. 28/95 (29.5%);p=0.02, **Hospital stay*:** 17.17± 3.23 vs.24.07 ±4 days; p<0.001
17. Sari 2011[55]	**Turkey**	**Participants:** Preterm infants<33w or birth weight<1500g **Intervention and dosage:** *L. sporogenes,* 0.35x10^9 CFU, once a day vs. No Probiotic **Duration of supplementation:** From first enteral feed until discharge **N=221** (Probiotics: 110, Controls: 111) **Type of milk**: EBM/ Formula **Type of delivery**: CS 67.3% vs. 75.7% **Primary outcomes: NEC ≥ Stage II:**6/110(5.5%) vs. 10/111(9%); p=0.447, **Death/NEC:** 9/110(8.2%) vs. 13/111(11.7%); p=0.515 **Other outcomes: LOS:** 29/110 (26.4%) vs 26/111 (23.4%);p=0.613, **Hospital stay**: 34.5 vs. 30 days; p=0.919, **Time to full feeds***: 17.3±8.7 vs. 18.3±9.8 days, p=0.438, **Feed intolerance:** 49/110(44.5%) vs. 70/111(63.1%);p=0.006
18. Serce 2013[56]	**Turkey**	**Participants:** Preterm infants<32 weeks and <1500g **Intervention and dosage:** *S. boulardii* 0.5x10^9 CFU twice daily vs. Placebo (Distilled Water)

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		Duration of supplementation: From the first enteral feed until discharge **N=208** (Probiotic: 104, Placebo: 104) **Type of milk:** EBM/ Formula **Type of Delivery:** CS 80.8% vs 88.5% **Primary outcomes: Stage ≥ 2 NEC:** 7/104(6.7%) vs. 7/104(6.7%); p=1, **LOS:** 19/104 (18.3%) vs. 25/104 (24.3%);p=0.29 **Other outcomes: Death:** 5/104(4.8%) vs. 4/104(3.8%);p=0.74, **Hospital stay^:** 39(28-60) days vs. 43(29-60) days; p=0.62
19. Shadkam 2015[57]	**Iran**	**Participants:** Preterm infants 28 to 32 weeks and 1000-1800g **Intervention and dose:** (*L. reuteri DSM 17938*.: $2.x10^7$ CFU) vs. distilled water **Duration of supplementation::** Twice daily started once infant reached 40ml/kg/day of feed till 120ml/kg/day of feed **N =60** (Probiotics: 30, Controls: 30) **Type of milk**: EBM/ formula milk **Type of delivery**: details NA **Primary outcome:** (Stage NS) NEC (2/30, 6.7% vs. 11/30, 36.7%); p=0.005 **Other outcomes: LOS:** 4/30(13.3%) vs. 10/30(33.4%); p=0.109**, TFEF*:** 12.83±4.26 vs. 16.78±6.66 days; p=0.01, **Mortality:** 1/30 (3.3%) vs. 2/30(6.7%); p=0.5
20.Tewari 2015[58]	**India**	**Participants:** Preterm infants <34 weeks (2 groups: extremely preterm/EPT: 27-30+6weeks and Very preterm/VPT: 31-33+6 weeks) **Intervention:** *Bacillus clausii* (2.4×10^9 spores per day) vs. Placebo **Duration of supplementation:** Commenced D5 in asymptomatic and D10 in symptomatic neonates and continued for 6 weeks/discharge/death/occurrence of LOS whichever was earlier **N=244** (Study: EPT: 61 and VPT:62) vs.(Placebo:121) **Type of milk:** EBM/PDHM **Type of delivery:** CS: EPT: 66% vs 59% and VPT: 58% vs. 60% **Primary outcome: Incidence of definite and probable LOS: Definite LOS:** EPT: 6/61(10%) vs. 8/59(14%); p=0.26, VPT: 2/62(3%) vs. 3/62(5%);p=0.39, **Probable LOS:** EPT: 8/61(12%) vs. 9/59(15%), VPT: 4/62(6%) vs. 5/62(7%) **Other outcomes: Death:** EPT: 8/61(13%) vs. 9/59(15%);p=0.84, VPT: 4/62(7%) vs. 5/62(8%);p=0.79, **NEC (≥stage 2):** EPT: 0/61 vs. 0/59, VPT: 0/62 vs. 0/62
21. Van Niekerk 2015[59]	**S. Africa**	**Participants:** Preterm infants<34 weeks and birth weight 500g to 1250g **Intervention and dosage:** Pro-52 (*L. rhamnosus GG and B. infantis*) ,$0.35x10^9$ CFU of each daily vs. Placebo (MCT oil) **Duration of supplementation:** From the first enteral feed till day 28 of life **N=184** (Probiotic: 91, Placebo: 93) **Type of milk:** EBM/ Formula **Type of Delivery:** CS 80.8% vs 88.5% **Primary outcome:** Impact of probiotic supplementation on the incidence and severity of NEC in premature VLBW infants that are exposed to HIV. **NEC:** 3/91(3.3%) vs 6/93(6.45%) **Other outcomes: LOS:** 15/91(16.5%) vs 10/93(10.8%)**, Death:** 5/91(5.5%) vs 6/93(6.45%), **TFEF*: HIV exposed:** 10.19±4.055 vs. 9.68 ± 3.46 days, p=0.56 and **HIV non-exposed:** 9.63± 2.42 vs. 11.14± 4.15 days, p=0.022
22.Yang 2011[60]	**China**	**Participants:** 62 preterm infants <37 weeks **Intervention:** *B. longum, L. acidophilus, Enterococcus fecalis* triple viable powder oral or nasal Bifico plus powder / capsules (probiotics powder / capsules), Shanghai Xinyi Pharmaceutical Co., Ltd.), 0.5×10^7 CFU twice daily of each **Duration of supplementation:** from commencement of feeds till 14 days of life **N=62** (Controls:31, Probiotics: 31) **Type of milk:** EBM/preterm formula **Type of Delivery:** NA

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		Primary outcomes: NEC incidence: 2/31 (6.45%) vs. 3/31 (9.68%) vs (No mention of criteria for NEC used) **Other outcomes: Sepsis, Mortality, TFEF:** NA
23. Xu 2015[62]	**China**	**Participants:** 125 neonates with a gestational age of 30-37 weeks and birth weight 1500-2500 g. **Intervention:** *S. boulardii* CNCM I-745 at a dose of 50 mg/kg (10^9 CFU) twice a day **Duration of supplementation:** 9-28 days (mean 25.3 days) **N=125** (Probiotic:63, Control:62), Analysis (Probiotic:51, Control:49) **Type of milk:** EBM/formula **Type of delivery:** NA **Primary outcome:** Weight gain was 16.14 ± 1.96 g/kg/day vs. 10.73 ± 1.77 g/kg/day; p<0.05 and Linear growth was 0.89 ± 0.04 cm/week vs 0.87 ± 0.04 cm/week; p=0.17 **Other outcome:** TFEF: 0.37 ± 0.13 vs 1.70 ± 0.45; p<0.01, maximal enteral feeding volume tolerated :128.44 ± 6.67 vs. 112.29 ± 7.24 ml/kg/day: p=0.03, and duration of hospitalization: 23.3 ± 1.6 vs. 28.0 ± 1.8; p=0.035

Abbreviations: L: Lactobacillus, B: Bifidobacterium, S: Saccharomyces, CFU: Colony Forming Unit, VLBW: Very Low Birth Weight, CS: *Caesarean section,* ***EBM:*** *expressed breast Milk,* ***EOS:*** *early onset sepsis,* ***EPT:*** *Extremely preterm,* ***LGG:*** *Lactobacillus rhamnosus GG (ATCC 53103) Gorbach and Goldin,* ***LOS:*** *Late onset sepsis,* ***LS-*** *Lower Segment,* ***LSCS:*** *Lower Segment Caesarean Section,* ***NA:*** *Data Not Available,* ***NEC:*** *Necrotizing enterocolitis,* ***NS:*** *not specified,* ***PDHM:*** *pasteurised donor human Milk,* ***PMA:*** *post menstrual age,* ***SCFA:*** *Short Chain Fatty Acid,* ***TFEF:*** *Time to full enteral feeds,* ***VD:*** *Vaginal delivery,* ***VPT:*** *very preterm*

- ***For all outcomes, results in the study/probiotic group are given first***
- ***^: median and interquartile range (25-75%), * : mean and SD***

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Table 2: Risk of Bias of the included RCTs

Author/ Year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Awad 2010	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Braga 2010	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dashti 2015	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Demirel 2013	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Deng 2010	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Dilli 2015	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dutta 2015	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Fernandez-Carrocera 2013	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hua 2014	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk
Huang 2009	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Oncel and Sari 2014	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Qiao 2012	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Rojas 2012	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Roy 2014	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Saengtawesin 2014	Low risk	Unclear risk	High risk	High risk	Low risk	Low risk	Unclear risk
Samanta 2008	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
Sari 2011	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shadkam 2015	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Serce 2013	Low risk	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Tewari 2015	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Van Niekerk 2014	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Yang 2011	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Xu 2015	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk

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Table 3: Results of the subgroup analysis (risk of bias)

Item	Number of studies	Sample size	RR (95% CI) (FEM)	RR (95% CI) (REM)	I^2 statistic
Definite NEC: Studies with low ROB on random sequence generation	14	3464	0.55 (0.40,0.74)	0.58 (0.42,0.81)	1%
Definite NEC: Studies with low ROB on allocation concealment	13	3035	0.48 (0.34, 0.66)	0.52 (0.33, 0.80)	29%
LOS: Studies with low ROB on random sequence generation	15	3466	0.85 (0.74, 0.97)	0.84 (0.72, 0.98)	18%
LOS: Studies with low ROB on allocation concealment	11	2839	0.86 (0.75, 0.99)	0.85 (0.74, 0.97)	6%
All cause mortality: Studies with low ROB on random sequence generation	14	3366	0.72 (0.57, 0.91)	0.75 (0.60, 0.95)	0%
All cause mortality: Studies with low ROB on allocation concealment	13	3073	0.76 (0.60, 0.96)	0.78 (0.62, 0.99)	0%

LOS: Late onset sepsis; RR: Relative Risk; CI: Confidence Interval; FEM: Fixed effects model; REM: Random effects model; ROB: Risk of bias

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Table 4: Results of the subgroup analysis

Item	Definite NEC			Late onset sepsis			All cause mortality		
	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)
RCTs with gestational age<32 weeks or birth weight<1500g	14 (2886)	0.51(0.37,0.70)	0.56(0.40,0.78)	11 (2470)	0.84(0.71,1.01)	0.84(0.68,1.04)	12 (2591)	0.75(0.61,0.93)	0.78(0.61,0.99)
RCTs: *Lactobacillus* was part of the supplementation	13 (2595)	0.45(0.32,0.64)	0.48(0.32,0.71)	12 (2979)	0.81(0.70,0.93)	0.79(0.64,0.97)	16 (3473)	0.70(0.56,0.89)	0.73(0.58,0.93)
RCTs: *Bifidobacterium* was part of the supplementation	11 (1716)	0.35(0.22,0.55)	0.38(0.23,0.63)	9 (1756)	0.76(0.64,0.89)	0.75(0.59,0.94)	12 (2173)	0.70 (0.52,0.93)	0.71 (0.49,1.03)
Single strain probiotic supplementation	11 (2727)	0.46(0.32,0.66)	0.46(0.32,0.66)	9 (2446)	0.86 (0.7,1.04)	0.83(0.67,1.03)	9 (2444)	0.70(0.52,0.94)	0.71 (0.53,0.96)
Multi strain probiotic supplementation	9 (1333)	0.45(0.28,0.73)	0.47(0.28,0.78)	8 (1556)	0.76(0.65,0.90)	0.75(0.59,0.96)	10 (1752)	0.76(0.56,1.03)	0.78 (0.54,1.13)

LOS: Late onset sepsis; RR: Relative Risk; CI: Confidence Interval; FEM: Fixed effects model; REM: Random effects model; ROB: Risk of bias

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Table 5: Summary of findings as per GRADE guidelines[38]

Outcome	Absolute risk					
	Estimate without probiotic supplementation	Corresponding risk estimate with probiotic supplementation	Relative effect (RR) 95% CI	Number of participants	Quality of Evidence GRADE	Comment
Late onset sepsis	358/1986 (18%)	308/1986 (14.5%)	0.80(0.71,0.91); p=0.0009, I^2=25%	3902	High	Ref note*
Mortality	176/2048 (8.6%)	137/2148 (6.4%)	0.73 (0.59,0.9); p=0.003, I^2=0%	4196	High	Ref note*
NEC	135/1957 (6.9%)	65/2065 (3.1%)	0.46 (0.34,0.61); p<0.00001, I^2 =19%	4022	High	Ref note*

*__Note:__ The evidence was deemed high in view of the large sample size, low risk of bias in majority (14/20) of the included studies, narrow confidence intervals around the effect size estimate, very low p value for effect size estimate and mild statistical heterogeneity.

Abbreviations: GRADE, grades of recommendation, assessment, development and evaluation; RR, risk ratio, CI: Confidence interval.

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Figure Legends

Figure1

Flow diagram of search strategy and study selection (January 2017)

Figure2

Forest plot: Effect of probiotics on definite (≥ stage II) NEC

Figure3

Forest plot: Effect of probiotics on late onset sepsis (LOS)

Figure4

Forest plot: Effect of probiotics on all cause mortality

Figure5

Forest plot: Effect of probiotics on time to full feeds (TFF)

Figure6

Funnel Plot assessing publication bias

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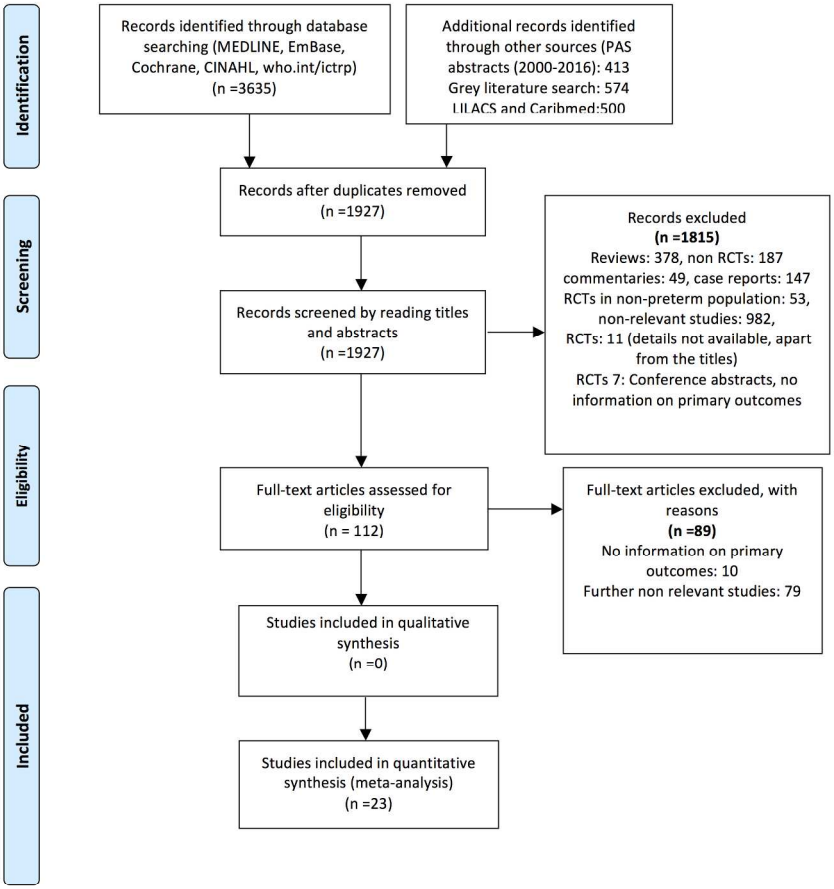
Figure 1; Flow diagram of search strategy and study selection (January 2017)

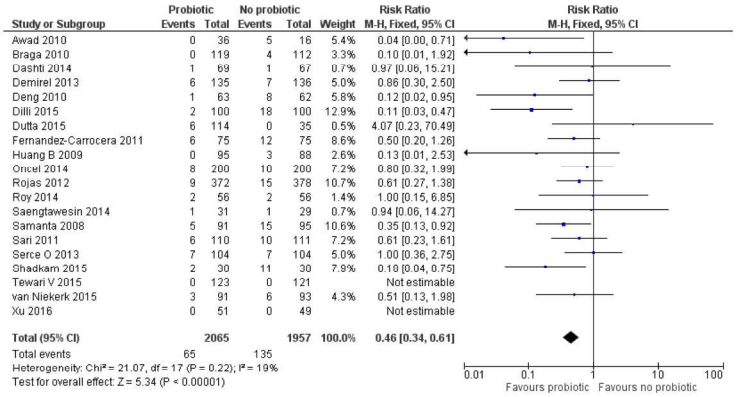


Flow diagram of search strategy and study selection (January 2017)

215x279mm (300 x 300 DPI)

Figure 2

Forest plot: Effect of probiotics on definite (≥ Stage II) NEC



Forest plot: Effect of probiotics on definite (≥ stage II) NEC

297x209mm (300 x 300 DPI)

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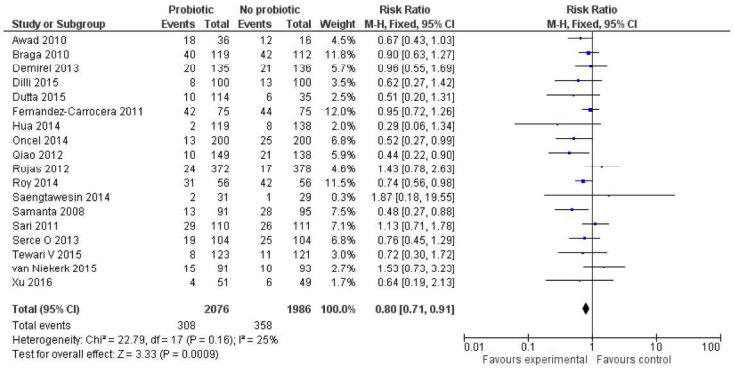
Figure 3

Forest plot: Effect of probiotics on late onset sepsis (LOS)



Forest plot: Effect of probiotics on late onset sepsis (LOS)

297x209mm (300 x 300 DPI)

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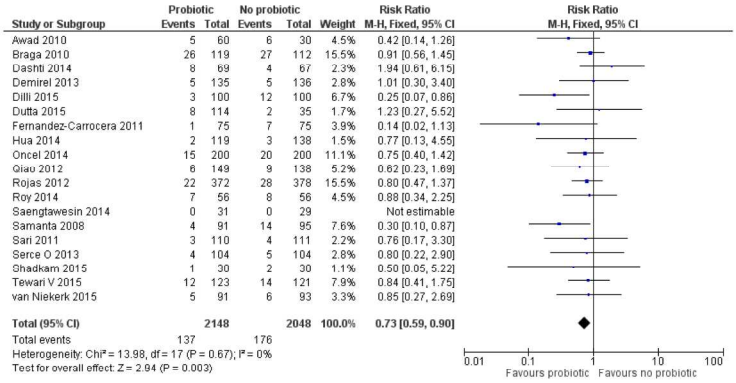
Figure 4

Forest plot: Effect of probiotics on all cause mortality



Forest plot: Effect of probiotics on all cause mortality

297x209mm (300 x 300 DPI)

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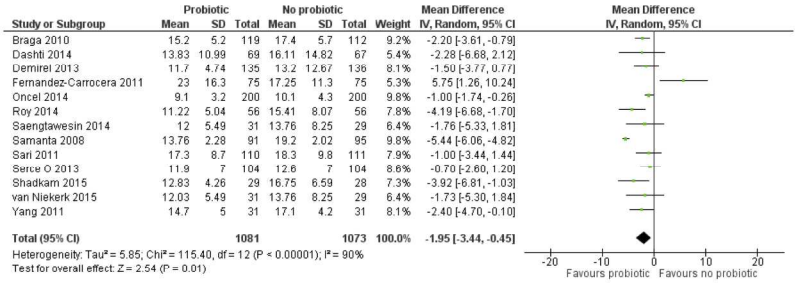
Figure 5

Forest plot: Effect of probiotics on time to full feeds (TFF)



Forest plot: Effect of probiotics on time to full feeds (TFF)

297x209mm (300 x 300 DPI)

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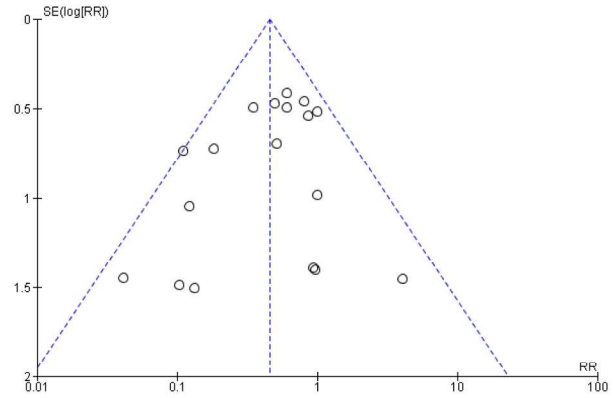
Figure 6
Funnel plot assessing publication bias



Funnel Plot assessing publication bias

297x209mm (300 x 300 DPI)

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Appendix 1: Search strategy

When searched: December 2016 and January 2017

PubMed:

- ((("Infant, Newborn"[Mesh]) OR ("Infant, Extremely Premature"[Mesh] OR "Infant, Premature"[Mesh])) OR ("Infant, Low Birth Weight"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh])) AND "Probiotics"[Majr]: **716**
- (("Infant, Extremely Premature"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh] OR "Infant, Small for Gestational Age"[Mesh] OR "Infant, Premature, Diseases"[Mesh] OR "Infant, Premature"[Mesh] OR "Infant, Newborn, Diseases"[Mesh] OR "Infant, Newborn"[Mesh] OR "Infant, Low Birth Weight"[Mesh])) AND (((("Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Saccharomyces"[Mesh]): **774**
- probiotics and preterm infants: **350**
- probiotics and low birth weight infants: **146**
- probiotics and sepsis:**321**
- probiotics and ELGAN(extremely low gestational age) infants: **7**
- probiotics and Necrotizing enterocolitis: **381**

EMBASE: (probiotics.mp. or probiotic agent)/AND **(**preterm infant.mp. OR prematurity/low birth weight infant.mp. OR low birth weight/ very low birth weight infant.mp. OR very low birth weight/extremely low birth weight infant.mp. OR extremely low birth weight/small for gestational age.mp. OR small for date infant OR ELGAN.mp OR extremely low gestational age neonate.mp): **711**

CINAHL: 113

Cochrane: 84 trials

who.int /ictrp (WHO International Clinical Trials Registry Platform): 26, Relevant: 17, Recruiting: 4 (2 of the relevant)

PAS 2000-2014: 187 (probiotics), 68 (Bifidobacteria), 137 (Lactobacillus/ Lactobacilli), Saccharomyces (15)

PAS 2015: 17 (probiotics), 6 (Bifidobacteria), 4 (Lactobacillus/ Lactobacilli), Saccharomyces (2)

Grey literature search: Using term “probiotics and preterm infants”

- **Ntis.gov/:** 42, Relevant: 0; **Opengrey.eu/:** 2, Relevant: 0; **Trove.nla.gov.au**: 495, Duplicates:253, Not relevant:242

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5,6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6,7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6,7 and appendix1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Figure 1
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7,8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7.8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2 for each meta-analysis.	8

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2-5
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9,10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 3,4
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11,12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11,12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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Benefits of probiotics in preterm neonates in low and medium income countries - a systematic review of randomised controlled trials

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Benefits of probiotics in preterm neonates in low and medium income countries - a systematic review of randomised controlled trials

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Word count: Abstract: 291, Text: 3213 (excluding tables)

Key words: *infant, newborn, necrotising enterocolitis, preterm neonates, sepsis, probiotics, review, surgical, supplementation, developing countries*

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Abstract

Objective: Although there is an overall reduction in under five mortality rate, the progress in reducing neonatal mortality rate has been very slow. Over the last 20 years, preterm births have steadily increased in low and medium income countries (LMIC) particularly in sub-Saharan Africa and South Asia. Preterm birth is associated with increased mortality and morbidity, particularly in LMICs. Based on systematic reviews of randomised controlled trials (RCT), many neonatal units in high income countries have adopted probiotics as standard of care for preterm neonates. We aimed to systematically review the safety and efficacy of probiotics in reducing mortality and morbidity in preterm neonates in LMICs.

Design: Systematic review and meta-analysis of RCTs.

Data sources: Medline, EMBASE, Cochrane Central Register of Controlled Trials, CINAHL (Cumulative Index of Nursing and Allied Health Literature), and E-abstracts from Pediatric Academic Society meetings and other paediatric and neonatal conference proceedings were searched in January 2017

Eligibility criteria: RCTs comparing probiotics vs. placebo/no probiotic in preterm neonates (gestation <37 weeks) conducted in LMICs.

Results: Total 23 (N=4783) RCTs from 4 continents and 10 LMICs, were eligible for inclusion in the meta-analysis using fixed effects model. The risk of necrotising enterocolitis (NEC≥ Stage II) [RR: 0·46(95% CI: 0·34, 0·61) p <0.00001, NNT: 25 [95% CI: 20, 50], late onset sepsis (LOS) [RR: 0·80(95% CI: 0·71, 0·91) p=0.0009, NNT: 25 [95% CI: 17, 100] and all-cause mortality [RR: 0·73(95% CI: 0·59, 0·90) p=0.003, NNT: 50 [95% CI: 25, 100] was significantly lower in probiotic supplemented neonates. The results were significant on random effects model analysis and after excluding studies with high risk of bias. No significant adverse effects were reported.

Conclusion: Probiotics have significant potential to reduce mortality and morbidity (e.g. NEC, LOS) in preterm neonates in LMICs

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Strengths and limitations of this study

- The strengths of our systematic review include its robust methodology, comprehensive nature, large sample size, and exclusive focus on RCTs of probiotics in preterm neonates in LMIC.
- The limitations include variations in the probiotic protocols in the included RCTs. Furthermore nearly 40% of the included trials carried a high risk of bias in many domains of assessment.

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Introduction

The UNICEF 2010 report showed that the global burden of under five mortality was reduced by one third compared to 1990s; however progress in reducing neonatal mortality has been slow. [1-3] Almost 40% of under five deaths occur during the neonatal period and majority of these deaths occur in Sub-Saharan Africa, South Asia, and Oceania. An estimated 98% of all neonatal deaths occur in low and medium income countries (LMIC). [4-6] Out of 135 million births each year, 3.1 million have died within the neonatal period and nearly 35% of these deaths occur in preterm neonates.[2,5] It may be perceived that prematurity is not a problem of LMICs. However, it is important to note that only 8.6% of preterm births occur in developed countries[5] Over the last 20 years, the number of preterm births has steadily increased to 9.1million as of 2010 in the regions of sub-Saharan Africa and South Asia. Preterm birth is associated with increased risk of mortality, and morbidity including late onset sepsis (LOS), necrotising enterocolitis (NEC), feeding difficulties, and long term neurodevelopmental impairment (NDI). [6-8] Although survival of preterm neonates has improved in some LMICs, morbidities such as NEC and LOS are still a major issue.[5,9-12] Considering the United Nation's millennium developmental goal (MDG-4), and the United Nation Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying 'Every Woman, Every Child initiative, Every Newborn Action plan' (ENAP), it is important to develop cost-effective simple strategies to reduce the mortality and morbidity associated with prematurity in LMICs.[13]

The World Health Organisation (WHO) defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".[14] Probiotics have been shown to significantly reduce the risk of NEC, all-cause mortality, LOS and facilitate feed tolerance in preterm very low birth weight (VLBW) neonates.[15-17] The mechanisms of benefits of probiotics include gut barrier enhancement, immune response

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modulation (e.g. TLR4 receptor, nuclear factor-B, inflammatory cytokines), and direct inhibition of gut colonisation by pathogens.[18-22] Many developed countries are already using probiotics routinely in preterm neonates for prevention of NEC.[23-32] It has been suggested that probiotics may have a role in LMICs for prevention, treatment of acute gastrointestinal diseases, particularly in children with HIV infection.[33-36] Given their simplicity and affordability, we aimed to systematically review the safety and efficacy of probiotics in reducing the risk of mortality and morbidity in preterm neonates in LMICs.

Methods

Guidelines from the Cochrane Neonatal Review Group (http://neonatal.cochrane.org/resources-review-authors),[37] Centre for Reviews and Dissemination (http://www.york.ac.uk/crd/guidance/),[38] and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [39] were followed for undertaking and reporting this systematic review and meta-analysis. Ethics approval was not required.

Eligibility criteria

Types of studies: Only randomised controlled trials (RCTs) were included in the review. Observational studies, narrative/systematic reviews, case reports, letters, editorials and commentaries were excluded, but read to identify potential additional studies.

Types of participants: Preterm neonates born at a gestational age (GA) <37 weeks or low birth weight (LBW: <2500 grams) or both (Same criteria as the Cochrane review, 2014).[15]

Setting: Only RCTs from LMICs were included. LMICs were defined as per the World Bank guidelines which include countries with gross national income (GNI) per capita of under $12736/year.[40]

Intervention and comparison: Enteral administration of probiotic supplement versus control (placebo/no probiotic).

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Outcomes: All-cause mortality, LOS (Positive blood/CSF culture on a sample collected 48-72 hours after birth), Definite NEC (Stage ≥II modified Bell staging)[41] and time to full enteral feeds (TFF: 120ml/kg/day).

Search strategy: The databases Medline searched via PubMed (www-ncbi-nlm-nih-gov, 1966-2017), EMBASE (Excerpta Medica dataBASE) via Ovid (http://ovidsp.tx.ovid.com, 1980-2017), Cochrane Central Register of Controlled Trials (www.thecochranelibrary.com, through January 2017), CINAHL (Cumulative Index of Nursing and Allied Health Literature) via OVID (http://ovidsp.tx.ovid.com, 1980- January 2017), and E-abstracts from the Pediatric Academic Society meetings (www.abstracts2view.com/pasall, 2000- January 2017) were searched in January 2017. Abstracts of other conference proceedings such as European Academy of Paediatric Societies (EAPS) and the British Maternal and Fetal Medicine Society were searched in EMBASE. 'Google Scholar' was searched for articles that might not have been cited in the standard medical databases. Grey literature was searched using the national technical information services (http://www.ntis.gov/), Open Grey (http://www.opengrey.eu/), and Trove (http://trove.nla.gov.au/). We have also searched LILACS and Caribmed via the BIREME/PAHO/WHO - Latin American and Caribbean Center on Health Sciences Information (http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/) using broad terminologies Probiotics OR Probiotic Or Bifidobacterium OR Bifidobacteria OR Lactobacillus OR Lactobacilli OR Saccharomyces. We also searched https://clinicaltrials.gov, http://www.who.int/ictrp/en/, and www.bioportfolio.com for ongoing RCTs. The reference lists of eligible studies and review articles were searched to identify additional studies. Reviewers SR, GJ and GD conducted the literature search independently. No language restriction was applied. The non-English studies were identified by reading the recent systematic reviews of probiotic supplementation for reducing the risk of NEC,[42,43] and from cross references of individual studies. Full texts of all non-English

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studies were obtained via University of Sydney and Department of New South Wales (NSW) health library. A research officer from the NSW Health, University of Sydney translated the articles. Attempts were made to contact the authors for additional data and clarification of methods. Only published data were used for those studies, where available.

PubMed was searched using the following terminology: ((("Infant, Newborn"[Mesh]) OR ("Infant, Extremely Premature"[Mesh] OR "Infant, Premature"[Mesh])) OR ("Infant, Low Birth Weight"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh])) AND "Probiotics"[Majr]. It was also searched using (("Infant, Extremely Premature"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh] OR "Infant, Small for Gestational Age"[Mesh] OR "Infant, Premature, Diseases"[Mesh] OR "Infant, Premature"[Mesh] OR "Infant, Newborn, Diseases"[Mesh] OR "Infant, Newborn"[Mesh] OR "Infant, Low Birth Weight"[Mesh])) AND ((("Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Saccharomyces"[Mesh]). The other databases were searched using similar terminologies. The detailed search terminology is given in appendix 1.

Study selection: The abstracts of citations obtained from the initial broad search were read independently by reviewers SR, GJ, and GD, to identify potentially eligible studies. Full-text articles of these studies were obtained and assessed for eligibility by reviewers SR, GJ and GD independently, using the predefined eligibility criteria. Differences in opinion were resolved by group discussion to reach consensus. Care was taken to ensure that multiple publications of the same study were excluded to avoid data duplication.

Data extraction: Reviewers GD, SR and GJ extracted the data independently using a data collection form designed for this review. Information about the study design and outcomes was verified by all reviewers. Discrepancies during the data extraction process were resolved by group discussion. We contacted authors for additional information/clarifications.

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Assessment of risk of bias (ROB): ROB was assessed using the Cochrane "Risk of Bias Assessment Tool".[44] Authors GD, SR and GJ independently assessed the ROB in all domains including random number generation, allocation concealment, blinding of intervention and outcome assessors, completeness of follow up, selectivity of reporting and other potential sources of bias. For each domain, the ROB was assessed as low, high or unclear risk based on the Cochrane Collaboration guidelines.

Data synthesis: Meta-analysis was conducted using Review Manager 5•3 (Cochrane Collaboration, Nordic Cochrane Centre). Fixed-effects model (FEM) (Mantel–Haenszel method) was used. Random-effects model (REM) analysis was conducted to recheck the results if there was significant heterogeneity on FEM. Effect size was expressed as risk ratio (RR) and 95% % confidence interval (CI).

Statistical heterogeneity was assessed by the χ^2 test, I^2 statistic and visual inspection of the forest plot (overlap of CIs). A p value <0.1 on χ^2 statistic was considered to indicate heterogeneity. An I^2 statistic values were interpreted as per the Cochrane handbook guidelines as follows: 0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity.[37] The risk of publication bias was assessed by visual inspection of the funnel plot.[45]

Subgroup analysis: a) Low ROB: random sequence generation and allocation concealment

b) Preterm neonates less than 34 weeks gestation or birth weight less than 1500g.; c) Where *Bifidobacterium* was part of the supplementation; d) Where *Lactobacillus* was part of the supplementation; e). Single strain probiotic were used and f) Multiple strain probiotics were used.

Summary of findings table: The key information concerning the quality of evidence, the magnitude of effect of the intervention and the sum of available data on the main outcome

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was presented in the 'summary of findings table' as per the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) guidelines.[44]

Funding source: Not required

Results

The literature search retrieved 1926 potential relevant citations. After carefully reviewing the abstracts, 1814 studies were excluded: Reviews: 378, observational studies: 187, commentaries: 49, case reports: 147, RCTs in adult and paediatric population: 53, and non-relevant studies: 982. Finally 23 RCTs (n=4783) conducted in 10 different LMICs in 4 continents were included in the meta-analysis.[12,46-67] The search strategy results are given in appendix 1. The flow diagram of study selection process is given in **figure 1**. The characteristics of the included studies are given in **table 1**. Out of the 23 included studies, Single-strain probiotics were used in 11 studies, whereas 12 used multiple strains. *Lactobacillus* was part of the supplementation in 13 studies; *Bifidobacterium* was part of the supplementation in 11 studies and saccharomyces in 3 studies. **(Table 1)**

ROB of included studies: A total of 14/23 (60%) included studies were judged to have low ROB for the domain of 'random sequence generation', and (56%) were considered to have low ROB for 'allocation concealment'. **(Table 2)**

Effect of probiotics on ≥Stage II (definite) NEC (Figure 2): Data on definite NEC was reported by 20 trials (N=4022).[12,46-53,55,56,58-65,67] A higher proportion of neonates in the control group developed definite NEC compared with the probiotic group [65/2065 (3.1%) vs. 135/1957 (6.9%)]. Meta-analysis using a FEM estimated a lower risk [RR: 0.46 (95% CI: 0.34, 0.61), p<0.00001] of NEC in the probiotic group. There was no significant heterogeneity (I^2=19%, p=0·22) among the trials. The numbers needed to treat (NNT) with probiotics to prevent one case of NEC was 25 (95% CI: 20, 50).

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Effect of probiotics on LOS (Figure 3): Data from 18 trials[12,46,47,49,51-54,56-62,64,65,67] (N=4062) showed that a higher proportion of neonates in the control group developed LOS compared with those in the probiotic group [308/2076 (14.5%) vs. 358/1986 (18%)]. Meta-analysis using a FEM estimated a lower risk [RR: 0.80 (95% CI: 0.71, 0.91), p=0.0009] of LOS in the probiotic group. There was no significant heterogeneity (I^2 =25%, p=0.16) among the trials. The NNT with probiotics to prevent one case of LOS was 25 (95% CI: 17, 50).

Effect of probiotics on all-cause mortality (Figure 4): Data from 19 trials (N=4196),[12,46-49 51-54 56-65] showed reduced risk of death due to all causes in the probiotic vs. control group [137/2148 (6.37%) vs. 176/2048 (8.59%)] Meta-analysis using a FEM estimated a lower risk [RR: 0.73(95% CI: 0.59, 0.90), p=0.003] of death in the probiotic group. No significant heterogeneity was noted between the trials (I^2 =0%, p=0.67). The NNT to prevent one death by probiotic supplement was 50 (95% CI: 25, 100).

Effect of probiotics on TFF (Figure 5) Meta-analysis of data (N=2154) from 13 trials [12,47-49, 53,56,59-63,65,66] showed significant reduction in TFF in the probiotics vs. control group [MD=-3.09 days (95% CI: -3.49, -2.69), p<0.00001]. However, there was significant heterogeneity (I^2= 90%, p< 0.00001) among the trials. These results were hence checked by using REM and remained significant [MD=-1.95 days (95% CI: -3.44, -0.45), p=0.01].

Subgroup analysis: The beneficial effects continued to be observed in studies a) Low ROB: random sequence generation and allocation concealment **(Table 3)** b). That only included infants with gestational age <34 weeks or birth weight <1500g; c) Where *Bifidobacterium* was part of the supplementation; d) Where *Lactobacillus* was part of the supplementation; e). Single strain probiotics were used and f) Multiple strain supplements were used; however, on REM meta-analysis, statistical significance was lost for some of these analyses **(Table 4).** The overall evidence according to GRADE guidelines is provided as a summary of findings table **(Table 5)**. The evidence was deemed high in view of the large sample size, low risk of

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bias in majority (14/20) of the included studies, narrow confidence intervals around the effect size estimate, very low p value for effect size estimate and mild statistical heterogeneity. Visual inspection of the funnel plot suggested that there was no publication bias **(Figure 6).**

Safety: None of the studies reported any significant adverse effects including probiotic sepsis.

Discussion

The results of our systematic review of 23 RCTs (N=4783) conducted in ten LMICs across four continents show that probiotic supplementation in preterm neonates (born <37 weeks) significantly reduces the risk of all-cause mortality, LOS and NEC in such a set up. The limitations of this review include variations in types of probiotics used in different studies and limitations of study qualities in few studies. The strengths of our systematic review include its robust methodology, comprehensive nature, and exclusive focus on RCTs of probiotics in preterm neonates in LMIC. The limitations of our review include the variations in the probiotic protocols in the included RCTs, and the fact that nearly 40% of the included trials carried a high risk of bias in many domains of assessment.

To our knowledge this is the first systematic review focusing on RCTs of probiotics in preterm neonates in LMICs. The summary findings as per GRADE guidelines confirm the high quality evidence it provides (Table 5). Our results are significant considering the United Nation's MDG4 and UN Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying Every Woman, Every Child initiative, Every Newborn Action plan (ENAP), and the burden of prematurity in LMICs.[4,5,13]

The incidence of prematurity is significantly increasing in LMICs compared to Europe or North America. There are issues related to reporting of preterm births and outcomes in LMICs.[68] However the studies funded by the WHO estimate 13 million preterm births/year in LMICs with 11 million (85%) of these being concentrated in Africa and Asia, ~0.5 million

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each in Europe and North America (excluding Mexico) and 0.9 million in Latin America and the Caribbean.[69] The highest rates (11.9%) and number (seven million) of preterm births were in Africa, and Asia respectively. Mortality and morbidities such as LOS, NEC and feeding difficulties are major issues in preterm neonates. Although specific data from LMICs is not available, ~one million preterm neonates die every year, predominantly due to sepsis, and long-term impairment in survivors is becoming an important issue. [70]

Consistent with our recent systematic review[71], our results show that probiotics reduced the risk of not only NEC and all-cause mortality but also of LOS in preterm neonates. [RR: 0.81 (95% CI: 0.71, 0.92), p=0.001]. The reduction of LOS by probiotics is important considering that neonatal sepsis is responsible for nearly a 3rd all neonatal deaths in LMICs.[19, 20,22,72-77]

It is important to note that the burden of NEC is as significant in LMICs as in high income countries. The incidence and severity of NEC is higher in LMICs and includes up to 15% cases of NEC totalis with ~100% mortality.[9,12] It occurs not only in VLBW and ELBW neonates but also in preterm neonates with higher birth weight. Lack of antenatal steroids and being small for gestational age (SGA) due to intrauterine growth restriction (IUGR) are known risk factors for NEC.[78] The reason for higher incidence of NEC in LMICs could include the higher numbers of preterm 'SGA-IUGR' births and limited coverage of antenatal steroids.[79,80] The NEC related mortality and morbidity is almost entirely due to progression of the illness from stage II to stage III. Management of surgical NEC is difficult in LMIC considering the limited resources. Primary prevention of NEC is therefore an important strategy for reducing the health burden of the condition in LMICs. Considering the effect size with regards to reduced risk of NEC, the benefits of probiotics in LMIC could not be overemphasised.

The issue of implementing probiotics for preterm neonates in LMICs is complex. The options include either reconfirming their safety and efficacy in large definitive RCTs in LMICs or

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adopting their routine use based on current evidence. Conducting large multicentre trials and accessing proven safe and effective probiotics is difficult, especially in resource limited set ups.[34] Apart from the significant budget the difficulties include regulatory hurdles, and logistics of importing a probiotic product, maintaining cold chain, and providing ongoing independent safety and quality control. However there are recent examples of large RCTs conducted successfully in community settings in LMICs.[81-83] Neonatal demographic characteristics such as gestation and IUGR, are an important issue in conducting RCTs in LMICs as they determine the risk of NEC, duration of probiotic supplementation, and the cost-benefit ratio. It is also important to note that many RCTs have used different probiotic/s and probiotic activity could be strain specific.

Knowledge of the pattern of gut colonisation in preterm neonates in a given set up is important before using probiotics for research or routine use. Dutta et al have reported abnormal intestinal colonization patterns in the first week of life in VLBW neonates in their level III neonatal intensive care unit in India.[52] On day one, 45% neonates had sterile guts, and by day three, all were colonized predominantly by E. coli, K. pneumoniae and Enterococcus fecalis. Only one isolate had lactobacilli and bifidobacteria were not detected during the study period. Formula feeding was associated with E. coli colonization. Results of completed [82] and ongoing trials such as NCT02552706 will be important.[83]

Probiotic sepsis, antibiotic resistance, and altered immune responses in the long run, are the potential adverse effects of probiotics in preterm neonates. Availability of killed or inactivated probiotic strains with clinically proven benefits may help not only in avoiding such adverse effects but also in avoiding the need to maintain the cold chain. Awad et al have compared the effect of oral killed (KP) versus living *lactobacillus acidophilus* (LP) in reducing the incidence of LOS and NEC in neonates.[46] Both LP and KP reduced the risk of NEC [Absolute risk reduction (ARR): 16%, 15%, respectively] and LOS (ARR: 18%)

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significantly compared with placebo. LOS and NEC was reduced significantly in neonates colonised versus not colonised by *lactobacillus* at day 7 (27.9 vs. 85.9%, 0 vs. 7.8%) and day 14 (48.7 vs. 91.7% for LOS and 0 vs. 20.8% for NEC). KP retained the benefits similar to LP on comparison between all groups. Given the global implications of these results, the benefits of inactivated/killed probiotics need to be assessed in further large definitive trials.

In summary our results indicate that probiotics are effective in significantly reducing the risk of all-cause mortality, LOS, and NEC in preterm VLBW neonates in LMICs. Considering the burden of death, disease (NEC, LOS), and suboptimal nutrition in preterm neonates in LMICs, cooperation between various stake holders (e.g. industry, scientists, regulatory agencies) is warranted to either develop or to improve access to high quality safe and effective probiotics in such set ups. Support from organisations such as the WHO is important in providing access to probiotics for the countries (e.g. sub-Saharan Africa) where most prematurity related deaths occur. Whether probiotics could be used for research and/or routine use in preterm neonates in LMICs will depend on the national health priorities, resources, and ethics.

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Authors' contributions: Dr Deshpande conceptualized and designed the study, performed an independent literature search, selected studies for inclusion, extracted and interpreted data, assessed risk of bias of included studies, handled the meta-analysis software, oversaw translation of manuscripts in the Chinese language and wrote the first and final drafts of the manuscript; Dr Athalye-Jape performed an independent literature search, selected studies for inclusion, contacted authors for additional information where necessary, extracted and

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interpreted the data, checked the data entered by Dr Deshpande on the meta-analysis software, assessed the risk of bias of included studies, and helped with the first and the final draft of the manuscript; Dr Rao performed an independent literature search, selected studies for inclusion, verified the extracted data, assessed risk of bias, interpreted data, and helped with the first and the final draft of the manuscript; Prof Patole supervised the project, acted as referee author in case of differences of opinion between the first 3 authors, interpreted the data, and supervised the first and approved the final versions of the manuscript; and all authors approved the final manuscript as submitted.

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Table 1: **Characteristics of included studies**

Study ID/	Location	Study characteristics
1. Awad 2010[40]	**Egypt**	**Participants:** All neonates admitted to nursery, 28-41 weeks and weight 1.1-4.3 kg **Intervention and dose:** Killed probiotic/KP (*L. acidophilus,* 6× 10^9 CFU) vs. Living probiotic/LP (*L. acidophilus,* 6× 10^9 CFU) vs. Placebo **Duration of supplementation:** commenced on D1, duration NA **N=150** (60 vs. 60 vs.30), **Preterm: 89** (37 vs. 36 vs. 16) **Type of milk:** details NA **Type of delivery:** Preterm CS: KP (57%) vs. LP (56%) vs. Placebo (75%) **Primary outcome: All outcomes for LP vs. KP vs. Controls:** Incidence of neonatal sepsis (18/36, 50% vs. 25/37,68% vs. 12/16, 75%; p=0.251) and NEC (0/36 vs. 1/37 vs.5/16; p=0.000) neonates and evaluation of efficacy of a KP **Other outcome: Mortality:** 4/36 (11.1%) vs. 12/37(32.4%) vs. 5/16(31.3%) p=0.076
2. Braga 2010[41]	**Brazil**	**Participants:** Preterm infants 750-1499g **Intervention and dose:** (*L. casei* + *B. breve* : 3.5x10^7 to 3.5x10^9 CFU) vs. No probiotic **Duration of supplementation::** Once daily from the second day of life until day 30 **N =231** (Probiotics: 119, Controls: 112) **Type of milk**: EBM/ PDHM **Type of delivery**: CS 53.8% vs. 49.1 % **Primary outcome:** ≥ Stage II NEC (0/119, 0% vs. 4/112, 3.6%) **Other outcomes: LOS:** 40/119 (33.6% vs 42/112 (37.5%)**, Mortality:** 26/119 (21.8%) vs. 27/112(24.1%)
3. Dashti 2014[42]	**Iran**	**Participants:** Preterm infants 700-1800 g **Intervention and dose:** (*L. acidophilus*, *L. rhamnosus*, *B. longum*, *L. bulgaricus*, *L. casei*, *S. thermophilus*, *B. breve,* and *Bifidobacterium*: total 1x10^9 CFU/sachet) vs. placebo powder **Duration of supplementation::** Once daily from first feed of life until discharge **N =136** (Probiotics: 69, Controls:67) **Type of milk**: EBM/ formula milk **Type of delivery**: CS 82.4% vs. 17.6 % **Primary outcome:** ≥ Stage II NEC (2/69, 2.9% vs. 1/67, 1.5%) **Other outcomes: Mortality:** 8/69 (11.6%) vs. 4/67(5.97%)
4. Demirel, Erdeve 2013[43]	**Turkey**	**Participants:** Preterm infants ≤32 w and ≤1500g **Intervention and dose: :** S. *boulardii,* 5x10^9CFU vs. no probiotic **Duration of supplementation:** NA **N=271** (Probiotic: 135, Controls: 136) **Type of milk**: EBM/ Formula **Type of delivery**: CS 77.7% vs. 83% **Primary outcome:** NEC ≥ stage 2(6/135, 4.4% vs. 7/136, 5.1%) p=1, **Mortality:**(5/135,3.7% vs. 5/136, 3.7%) p=1 **Other outcomes: LOS:** 20/135 (14.9%) vs 21/136 (15.4%) p=0.906**, Feed intolerance:**30/135(22.2%) vs. 62/136(46%), p<0.001
5. Deng 2010[44]	**China**	**Participants:** 125 preterm infants, <37 weeks, <2500 g at birth **Intervention and dose:** *B. longum, L. acidophilus, Enterococcus fecalis*, triple viable powder oral or nasal Bifico plus powder / capsules. For birth weight <1500 g: 0.33× 10^7 CFU of each probiotic twice daily and >1500 g: 0.5× 10^7 of each probiotic twice daily, Control: sterile warm water **Duration of supplementation:** commenced from first feed till 14 days of life

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		N=125 (**62** Controls 33.2 ± 2.3 weeks vs **63** Probiotic group 32.4 ± 2.8 weeks), **Type of Milk:** EBM/preterm formula **Type of Delivery:** NA **Primary outcome: NEC: Controls**: Bell Stage I (1/62, 1.6%), Bell Stage II (4/62, 6.5%), Bell Stage III (4/62, 6.5%) vs T**reatment** Bell Stage I (1/63,1.6%), Bell Stage II (1/63, 1.6%) **Other outcomes: LOS, Mortality:** NA
6. Dilli 2015[45]	**Turkey**	**Participants:** VLBW infants with a gestation of <32w and birth weight<1500g **Intervention and dose:** *B. lactis* (5x10^9 CFU) vs. Placebo (maltodextrin) **Duration of supplementation:** From day 8 of life, once daily until discharge or a maximum of 8 weeks **N=200** (Probiotic 100, Placebo: 100) **Type of milk:** EBM/Formula **Type of delivery: CS**: 35/100 (35%) vs. 37/100 (37%) **Primary outcome: NEC (≥stage 2):**2/100(2%) vs.18/100 (18%), p<0.001 **Other outcomes: LOS:** 8/100 (8%) vs 13/100 (13%), p=0.6, Mortality: 3/100 (3%) vs. 12/100 (12%), p=0.003, Time to full enteral feeds^ (150ml/kg/day): 18(14-23) days vs. 25(15-37) days, p<0.001
7. Dutta 2015[46]	**India**	**Participants:** Preterm infants 27-33 weeks gestation **Intervention:** High dose (10 billion CFU: *L. acidophilus, L. rhamnosus, B. longum, S. boulardii*) **vs.** Low dose (1 billion CFU*: L. acidophilus, L. rhamnosus, B. longum, S. boulardii*) vs. Placebo (potato starch, maltodextrin) **Duration of supplementation: Probiotic groups: (A)**: High dose for 21 days, (C): Low dose for 21 days, (B): High dose short course (D1-D14 and D15-D21) **N: Probiotic (114)** vs. **Placebo (35)** **Type of milk:** EBM /formula **Type of delivery:** Probiotic group vs. Placebo: SVD (69% vs. 60%), CS: data NA **Primary outcome: Stool colonisation rates on D14, D21, D28 with 3 different probiotic regimens** (*Lactobacillus* and *Bifidobacterium* colonisation was significantly higher in groups A, B, and C vs. placebo respectively. Groups A, B, and C did not differ from each other. There were trends toward more CFU of *Lactobacillus* and *Bifidobacterium* per ml of stool in group A vs. B and B vs. C. Groups A and B and spontaneous preterm labor (SPL) independently predicted high Lactobacillus counts on day 28; groups A, B, and C and SPL predicted high *Bifidobacterium* counts) **Other outcomes: LOS:** 10/114 (8.8%) vs. 6/35 (17.1%), p=0.14, **Mortality**:8/114(7%) vs.2/35(12.7%), p=0.85, **NEC (≥stage 2):** 6/114(5.3%) vs 0/35(0%), p=0.35
8. Fernandez-Carrocera 2013[47]	**Mexico**	**Participants:** Preterm infants<1500g **Intervention and dosage:** Multispecies probiotic product **(***L. acidophilus +L. rhamnosus +L. casei+ L. plantarum+ B. infantis+ S. thermophilus)* vs. No probiotic **Duration of supplementation:** From the day of commencement of enteral feeds, once daily. Actual Duration: NA **N=150** (Probiotics:75, Controls: 75) **Type of milk:** EBM/ Formula **Type of delivery:** data not available **Primary outcome:**≥ Stage 2 NEC: 6/75(8%) vs 12/75(16%), p=0.142 **Other outcomes: LOS:** 42/75 (56%) vs 44/75 (58.7%), p=NA, **Mortality:** 1/75(1.3%) vs. 7/75(9.3%), p=0.063
9. Hua 2014[48]	**China**	**Participants:** Preterm infants<37 weeks **Intervention and dosage:** Probiotic Jin Shuang QI (*L. acidophilus, S. thermophilus, Bifidobacterium*) 5 x 10^7 CFU/day. Vs no probiotic **Duration of supplementation:** From the day of commencement of enteral feeds, once daily. Duration of Supplementation: not clear **N=257** (Probiotics:119, Controls: 138)

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		Type of milk: EBM/ Formula **Type of delivery**: CS 55.5% vs 64.5% **Primary outcome: Stool colonisation by drug resistant bacteria** (No difference in both groups, p>0.05) **Other outcome: LOS:** 2/119(1.7%) vs. 8/138 (5.8%); p-0.168, **NEC (stage NS):** 0/119 vs. 2/138; p=0.501,**Mortality:** 2/119 vs. 2/138
10. Huang 2009[49]	**China**	**Participants:** Preterm infants 28-32 weeks and <1500g **Intervention and dosage:** *Bifidobacterium* (50 million live bacteria/capsule) 0.25x10^8 live bacteria oral/nasally twice daily vs. non-treatment (control) **Duration of supplementation:** From 7 days till 14 days of age **N=183** (Probiotic: 95, Control: 88) **Type of milk:** Not stated **Type of Delivery:** NA **Primary outcomes: NEC:** 2/95 (2.1%), both Bell's stage 1 vs. 9/88 (10.23%): Bell's stage 1:6, stage 2:2, stage 3:1 (p<0.01), Body mass changes/**Weight gain*:** Probiotic group: 8.109 ± 2. 127 g vs. Control group 6. 489 ± 2. 327 g (p<0.01) **Other outcomes: LOS, Death, TFF:** NA**, gut colonisation:** Post 7d of treatment, the two groups' intestinal bacteria and bacteria ratio of the total number of cocci and rods, the differences were statistically significant (P <0. 01). Rod bacteria ratio before and after preventive treatment groups showed no significant difference (P> 0 05.); in the control group rod bacteria ratio difference was statistically significant (P <0. 01)
11. Oncel 2014[50]	**Turkey**	**Participants:** Preterm Infants≤32w and <1500g **Intervention and dosage:** *L. reuteri DSM 17938* in oil based suspension,1x10^8 CFU/day vs Placebo (Oil based suspension without probiotics) **Duration of supplementation:** From the time of first enteral feeds until discharge **N=400** (Probiotics: 200, Placebo: 200) **Type of milk:** EBM/Preterm formula, **Type of delivery**: CS 75% vs. 76% **Primary outcome: Probiotics vs. Controls: ≥ Stage 2 NEC or death:** 20/200(10%) vs. 27/200(13.5%);p=0.27, **NEC (≥stage 2):**8/200(4%) vs.10/200(5%);p=0.63 **Other outcomes: Late Onset Sepsis:** 13/200 (6.5%) vs 25/200 (12.5%);p=0.041, **Time to full feeds*:**9.1±3.2 vs. 10.1±4.3 days; p=0.006, **Hospital stay^:**38(10-131) vs 46(10-180) days; p=0.022**, Feed intolerance:**56/200(28%) vs. 79/200(39.5%);p=0.015
12.Qiao 2012[51]	**China**	**Participants:** Preterm 28-34 weeks GA, >1000 g, <72 h life **Intervention:** *Bifidobacterium, Lactobacillus, Streptococcus thermophiles*, 0.5g per bag **Duration of supplementation:** 0.5 bag three times daily for 3 days after admission to hospital **N=287** (Probiotic: 149 vs Control 138) **Type of milk:** Not stated **Type of Delivery:** No Stats on CS/type of delivery **Primary outcomes:** Time to full oral feeds (7.3 d vs 16.9 d); p<0.05, time to full enteral nutrition (9.8 d vs 16.9 d); p<0.05, LOS (6.7% vs 15.2%); p<0.05, NEC (3.4% vs 10.9%); p<0.05, hospitalisation time (25.0 d vs 30.8 d); p:NA, Mortality*: (6.0 ± 4.0)% and (9.0 ± 6. 5)%;p>0.05
13. Rojas 2012[52]	**Columbia**	**Participants:** Preterm Infants≤2000g **Intervention and dosage:** *L. reuteri DSM 17938*, 1x10^8 CFU, once daily vs Placebo (Oil based suspension without probiotics) **Duration of supplementation:** Commenced within 48 hours of life. Duration: NA **N=750** (Probiotics:372, Placebo:378) **Type of milk:** EBM/Formula **Type of delivery**: VD non-instrumental: 16% (Study) vs. 17% (Placebo), VD instrumental: 0% (Study) vs. 0.5% (Placebo), Elective CS: 18% (Study) vs. 17% (Placebo), Non Elective CS 65% (Study) vs. 65% (Placebo)

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		Primary outcome: Nosocomial infection and mortality:57/372(15.3%) vs. 67/378(17.7%);p=0.38**, Death:** 22/372(5.9%) vs. 28/378(7.4%);p=0.41 **Other outcomes: LOS:** 24/372 (6.5%) vs 17/378 (4.5%);p=0.24, **Duration of hospitalisation**^: 20(11-33) vs. 20(11-38) days; p=0.53
14. Roy 2014[53]	**India**	**Participants:** Preterm infants<37w and birth weight<2500g **Intervention and dosage:** Half of the one gram sachet that contained *L. acidophilus* $1.25x10^9$ + *B. longum* $0.125x10^9$ + *B. bifidum* $0.125x10^9$ + *B. lactis* $1x10^9$ vs. Sterile water **Duration of supplementation**: Commenced within 72 hours of birth for six weeks or until discharge **N=112** (Probiotics: 56, Placebo: 56) **Type of milk**: EBM **Type of delivery**: CS 83.9% vs. 76.8% **Primary outcome**: **Enteric fungal colonisation*:** 3.03± 2.33 $\times10^5$ CFU vs. 3± 1.5×10^5; p=0.03 and **LOS (bacterial and fungal):** 31/56(55.4%) vs. 42/56(75%); p=0.02 **Other outcome: TFEF*:**11.22± 5.04 vs. 15.41± 8.07 days; p=0.016
15.Saengtawesin 2014[54]	**Thailand**	**Participants:** Preterm(<34 w) and VLBW (<1500g) infants **Intervention and dosage:** Probiotic mixture [*L. acidophilus* + *B. bifidum* each 1 $x10^9$ CFU/250mg], 125mg/kg twice daily vs. No probiotic **Duration of supplementation:** NA **N=60** (Probiotics: 31, Controls:29) **Type of milk:** EBM/preterm formula **Type of Delivery:** CS 67.7% vs. 62% **Primary outcome: NEC≥ stage 2:** 1 (3.2%) vs 1 (3.4%); p=0.74 **Other outcomes: LOS:** 2(6.45%) vs. 1(3.44%); p=0.53, **TFEF*:** 12.03 ± 5.49 days vs. 13.76 ± 8.25 days (p = 0.64).
16. Samanta 2009[12]	**India**	**Participants:** Preterm(<32 w) and VLBW (<1500g) infants **Intervention and dosage:** Probiotic mixture [*B. infantis* + *B. bifidum* + *B. longum* + *L. acidophilus*, each $2.5x10^9$ CFU], administered twice daily vs. No probiotic **Duration of supplementation:** NA **N=186** (Probiotics: 91, Controls: 95) **Type of milk:** EBM **Type of Delivery:** CS 46.15% vs 49.47% **Primary outcomes: Incidence of NEC(≥stage 2):**5/91(1.1%) vs.15/95(15.8%);p=0.042**, Death due to NEC:** Overall death: 4/91(4.4%) vs. 14/95(14.7%); p=0.032**, Feed tolerance: Time to full feeds*:** 13.76 ± 2.28 vs. 19.2 ± 2.02 days; p<0.001 **Other outcomes: LOS:** 13/91 **(**14.3%) vs. 28/95 (29.5%);p=0.02, **Hospital stay*:** 17.17± 3.23 vs.24.07 ±4 days; p<0.001
17. Sari 2011[55]	**Turkey**	**Participants:** Preterm infants<33w or birth weight<1500g **Intervention and dosage:** *L. sporogenes,* $0.35x10^9$ CFU, once a day vs. No Probiotic **Duration of supplementation:** From first enteral feed until discharge **N=221** (Probiotics: 110, Controls: 111) **Type of milk**: EBM/ Formula **Type of delivery**: CS 67.3% vs. 75.7% **Primary outcomes: NEC ≥ Stage II:**6/110(5.5%) vs. 10/111(9%); p=0.447, **Death/NEC:** 9/110(8.2%) vs. 13/111(11.7%); p=0.515 **Other outcomes: LOS:** 29/110 (26.4%) vs 26/111 (23.4%);p=0.613, **Hospital stay**: 34.5 vs. 30 days; p=0.919, **Time to full feeds***: 17.3±8.7 vs. 18.3±9.8 days, p=0.438, **Feed intolerance:** 49/110(44.5%) vs. 70/111(63.1%);p=0.006
18. Serce 2013[56]	**Turkey**	**Participants:** Preterm infants<32 weeks and <1500g **Intervention and dosage:** *S. boulardii* $0.5x10^9$ CFU twice daily vs. Placebo (Distilled Water)

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		Duration of supplementation: From the first enteral feed until discharge **N=208** (Probiotic: 104, Placebo: 104) **Type of milk:** EBM/ Formula **Type of Delivery:** CS 80.8% vs 88.5% **Primary outcomes: Stage ≥ 2 NEC:** 7/104(6.7%) vs. 7/104(6.7%); p=1, **LOS:** 19/104 (18.3%) vs. 25/104 (24.3%);p=0.29 **Other outcomes: Death:** 5/104(4.8%) vs. 4/104(3.8%);p=0.74, **Hospital stay^:** 39(28-60) days vs. 43(29-60) days; p=0.62
19. Shadkam 2015[57]	**Iran**	**Participants:** Preterm infants 28 to 32 weeks and 1000-1800g **Intervention and dose:** (*L. reuteri DSM 17938*.: $2.x10^7$ CFU) vs. distilled water **Duration of supplementation::** Twice daily started once infant reached 40ml/kg/day of feed till 120ml/kg/day of feed **N =60** (Probiotics: 30, Controls: 30) **Type of milk**: EBM/ formula milk **Type of delivery**: details NA **Primary outcome: (**Stage NS) NEC (2/30, 6.7% vs. 11/30, 36.7%); p=0.005 **Other outcomes: LOS:** 4/30(13.3%) vs. 10/30(33.4%); p=0.109**, TFEF*:** 12.83±4.26 vs. 16.78±6.66 days; p=0.01, **Mortality:** 1/30 (3.3%) vs. 2/30(6.7%); p=0.5
20.Tewari 2015[58]	**India**	**Participants:** Preterm infants <34 weeks (2 groups: extremely preterm/EPT: 27-30+6weeks and Very preterm/VPT: 31-33+6 weeks) **Intervention:** *Bacillus clausii* (2.4×10^9 spores per day) vs. Placebo **Duration of supplementation:** Commenced D5 in asymptomatic and D10 in symptomatic neonates and continued for 6 weeks/discharge/death/occurrence of LOS whichever was earlier **N=244** (Study: EPT: 61 and VPT:62) vs.(Placebo:121) **Type of milk:** EBM/PDHM **Type of delivery:** CS: EPT: 66% vs 59% and VPT: 58% vs. 60% **Primary outcome: Incidence of definite and probable LOS: Definite LOS:** EPT: 6/61(10%) vs. 8/59(14%); p=0.26, VPT: 2/62(3%) vs. 3/62(5%);p=0.39, **Probable LOS:** EPT: 8/61(12%) vs. 9/59(15%), VPT: 4/62(6%) vs. 5/62(7%) **Other outcomes: Death:** EPT: 8/61(13%) vs. 9/59(15%);p=0.84, VPT: 4/62(7%) vs. 5/62(8%);p=0.79, **NEC (≥stage 2):** EPT: 0/61 vs. 0/59, VPT: 0/62 vs. 0/62
21. Van Niekerk 2015[59]	**S. Africa**	**Participants:** Preterm infants<34 weeks and birth weight 500g to 1250g **Intervention and dosage:** Pro-52 **(***L. rhamnosus GG and B. infantis*) ,$0.35x10^9$ CFU of each daily vs. Placebo (MCT oil) **Duration of supplementation:** From the first enteral feed till day 28 of life **N=184** (Probiotic: 91, Placebo: 93) **Type of milk:** EBM/ Formula **Type of Delivery:** CS 80.8% vs 88.5% **Primary outcome:** Impact of probiotic supplementation on the incidence and severity of NEC in premature VLBW infants that are exposed to HIV. **NEC:** 3/91(3.3%) vs 6/93(6.45%) **Other outcomes: LOS:** 15/91(16.5%) vs 10/93(10.8%)**, Death:** 5/91(5.5%) vs 6/93(6.45%), **TFEF*: HIV exposed:** 10.19±4.055 vs. 9.68 ± 3.46 days, p=0.56 and **HIV non-exposed:** 9.63± 2.42 vs. 11.14± 4.15 days, p=0.022
22.Yang 2011[60]	**China**	**Participants:** 62 preterm infants <37 weeks **Intervention:** *B. longum, L. acidophilus, Enterococcus fecalis* triple viable powder oral or nasal Bifico plus powder / capsules (probiotics powder / capsules), Shanghai Xinyi Pharmaceutical Co., Ltd.), 0.5×10^7 CFU twice daily of each **Duration of supplementation:** from commencement of feeds till 14 days of life **N=62** (Controls:31, Probiotics: 31) **Type of milk:** EBM/preterm formula **Type of Delivery:** NA

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		Primary outcomes: NEC incidence: 2/31 (6.45%) vs. 3/31 (9.68%) vs (No mention of criteria for NEC used) **Other outcomes: Sepsis, Mortality, TFEF:** NA
23. Xu 2015[62]	**China**	**Participants:** 125 neonates with a gestational age of 30-37 weeks and birth weight 1500-2500 g. **Intervention:** *S. boulardii* CNCM I-745 at a dose of 50 mg/kg (10^9 CFU) twice a day **Duration of supplementation:** 9-28 days (mean 25.3 days) **N=125** (Probiotic:63, Control:62), Analysis (Probiotic:51, Control:49) **Type of milk:** EBM/formula **Type of delivery:** NA **Primary outcome:** Weight gain was 16.14 ± 1.96 g/kg/day vs. 10.73 ± 1.77 g/kg/day; p<0.05 and Linear growth was 0.89 ± 0.04 cm/week vs 0.87 ± 0.04 cm/week; p=0.17 **Other outcome:** TFEF: 0.37 ± 0.13 vs 1.70 ± 0.45; p<0.01, maximal enteral feeding volume tolerated :128.44 ± 6.67 vs. 112.29 ± 7.24 ml/kg/day: p=0.03, and duration of hospitalization: 23.3 ± 1.6 vs. 28.0 ± 1.8; p=0.035

Abbreviations: L: Lactobacillus, B: Bifidobacterium, S: Saccharomyces, CFU: Colony Forming Unit, VLBW: Very Low Birth Weight, CS: *Caesarean section,* ***EBM:*** *expressed breast Milk,* ***EOS:*** *early onset sepsis,* ***EPT:*** *Extremely preterm,* ***LGG:*** *Lactobacillus rhamnosus GG (ATCC 53103) Gorbach and Goldin,* ***LOS:*** *Late onset sepsis,* ***LS-*** *Lower Segment,* ***LSCS:*** *Lower Segment Caesarean Section,* ***NA:*** *Data Not Available,* ***NEC:*** *Necrotizing enterocolitis,* ***NS:*** *not specified,* ***PDHM:*** *pasteurised donor human Milk,* ***PMA:*** *post menstrual age,* ***SCFA:*** *Short Chain Fatty Acid,* ***TFEF:*** *Time to full enteral feeds,* ***VD:*** *Vaginal delivery,* ***VPT:*** *very preterm*

- ***For all outcomes, results in the study/probiotic group are given first***
- ***^: median and interquartile range (25-75%), * : mean and SD***

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Table 2: Risk of Bias of the included RCTs

Author/ Year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Awad 2010	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Braga 2010	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dashti 2015	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Demirel 2013	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Deng 2010	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Dilli 2015	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dutta 2015	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Fernandez-Carrocera 2013	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hua 2014	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk
Huang 2009	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Oncel and Sari 2014	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Qiao 2012	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Rojas 2012	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Roy 2014	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Saengtawesin 2014	Low risk	Unclear risk	High risk	High risk	Low risk	Low risk	Unclear risk
Samanta 2008	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
Sari 2011	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shadkam 2015	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Serce 2013	Low risk	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Tewari 2015	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Van Niekerk 2014	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Yang 2011	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Xu 2015	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk

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Table 3: Results of the subgroup analysis (risk of bias)

Item	Number of studies	Sample size	RR (95% CI) (FEM)	RR (95% CI) (REM)	I^2 statistic
Definite NEC: Studies with low ROB on random sequence generation	14	3464	0.55 (0.40,0.74)	0.58 (0.42,0.81)	1%
Definite NEC: Studies with low ROB on allocation concealment	13	3035	0.48 (0.34, 0.66)	0.52 (0.33, 0.80)	29%
LOS: Studies with low ROB on random sequence generation	15	3466	0.85 (0.74, 0.97)	0.84 (0.72, 0.98)	18%
LOS: Studies with low ROB on allocation concealment	11	2839	0.86 (0.75, 0.99)	0.85 (0.74, 0.97)	6%
All-cause mortality: Studies with low ROB on random sequence generation	14	3366	0.72 (0.57, 0.91)	0.75 (0.60, 0.95)	0%
All-cause mortality: Studies with low ROB on allocation concealment	13	3073	0.76 (0.60, 0.96)	0.78 (0.62, 0.99)	0%

LOS: Late onset sepsis; RR: Relative Risk; CI: Confidence Interval; FEM: Fixed effects model; REM: Random effects model; ROB: Risk of bias

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Table 4: Results of the subgroup analysis

Item	Definite NEC			Late onset sepsis			All cause mortality		
	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)
RCTs with gestational age<32 weeks or birth weight<1500g	14 (2886)	0.51(0.37,0.70)	0.56(0.40,0.78)	11 (2470)	0.84(0.71,1.01)	0.84(0.68,1.04)	12 (2591)	0.75(0.61,0.93)	0.78(0.61,0.99)
RCTs: *Lactobacillus* was part of the supplementation	13 (2595)	0.45(0.32,0.64)	0.48(0.32,0.71)	12 (2979)	0.81(0.70,0.93)	0.79(0.64,0.97)	16 (3473)	0.70(0.56,0.89)	0.73(0.58,0.93)
RCTs: *Bifidobacterium* was part of the supplementation	11 (1716)	0.35(0.22,0.55)	0.38(0.23,0.63)	9 (1756)	0.76(0.64,0.89)	0.75(0.59,0.94)	12 (2173)	0.70 (0.52,0.93)	0.71 (0.49,1.03)
Single strain probiotic supplementation	11 (2727)	0.46(0.32,0.66)	0.46(0.32,0.66)	9 (2446)	0.86 (0.7,1.04)	0.83(0.67,1.03)	9 (2444)	0.70(0.52,0.94)	0.71 (0.53,0.96)
Multi strain probiotic supplementation	9 (1333)	0.45(0.28,0.73)	0.47(0.28,0.78)	8 (1556)	0.76(0.65,0.90)	0.75(0.59,0.96)	10 (1752)	0.76(0.56,1.03)	0.78 (0.54,1.13)

LOS: Late onset sepsis; RR: Relative Risk; CI: Confidence Interval; FEM: Fixed effects model; REM: Random effects model; ROB: Risk of bias

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Table 5: Summary of findings as per GRADE guidelines[38]

Outcome	Absolute risk					
	Estimate without probiotic supplementation	Corresponding risk estimate with probiotic supplementation	Relative effect (RR) 95% CI	Number of participants	Quality of Evidence GRADE	Comment
Late onset sepsis	358/1986 (18%)	308/1986 (14.5%)	0.80(0.71,0.91); p=0.0009, I^2=25%	3902	High	Ref note*
Mortality	176/2048 (8.6%)	137/2148 (6.4%)	0.73 (0.59,0.9); p=0.003, I^2=0%	4196	High	Ref note*
NEC	135/1957 (6.9%)	65/2065 (3.1%)	0.46 (0.34,0.61); p<0.00001, I^2 =19%	4022	High	Ref note*

*__Note:__ The evidence was deemed high in view of the large sample size, low risk of bias in majority (14/20) of the included studies, narrow confidence intervals around the effect size estimate, very low p value for effect size estimate and mild statistical heterogeneity.

Abbreviations: GRADE, grades of recommendation, assessment, development and evaluation; RR, risk ratio, CI: Confidence interval.

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Figure Legends

Figure1

Flow diagram of search strategy and study selection (January 2017)

Figure2

Forest plot: Effect of probiotics on definite (≥ Stage II) NEC

Figure3

Forest plot: Effect of probiotics on late onset sepsis (LOS)

Figure4

Forest plot: Effect of probiotics on all-cause mortality

Figure5

Forest plot: Effect of probiotics on time to full feeds (TFF)

Figure6

Funnel plot assessing publication bias

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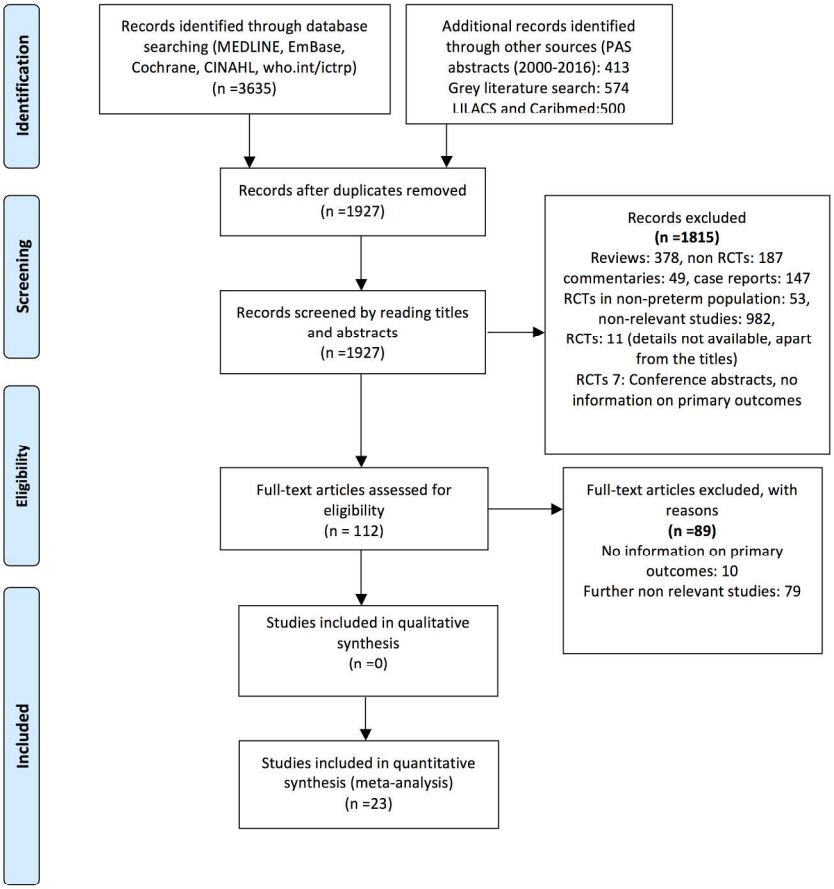
Figure 1; Flow diagram of search strategy and study selection (January 2017)

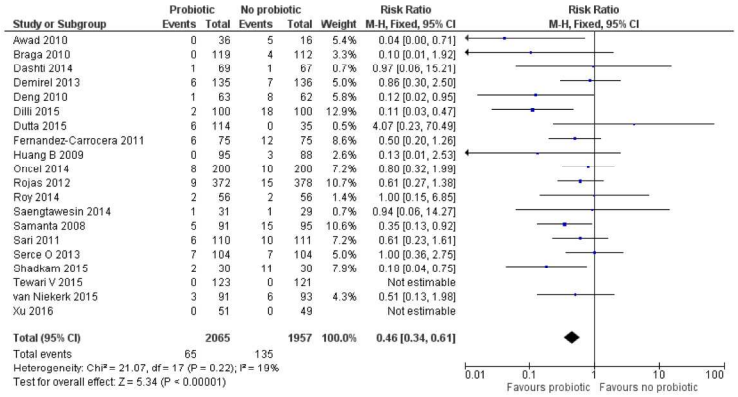


Flow diagram of search strategy and study selection (January 2017)

215x279mm (300 x 300 DPI)

Figure 2

Forest plot: Effect of probiotics on definite (≥ Stage II) NEC



Forest plot: Effect of probiotics on definite (≥ stage II) NEC

297x209mm (300 x 300 DPI)

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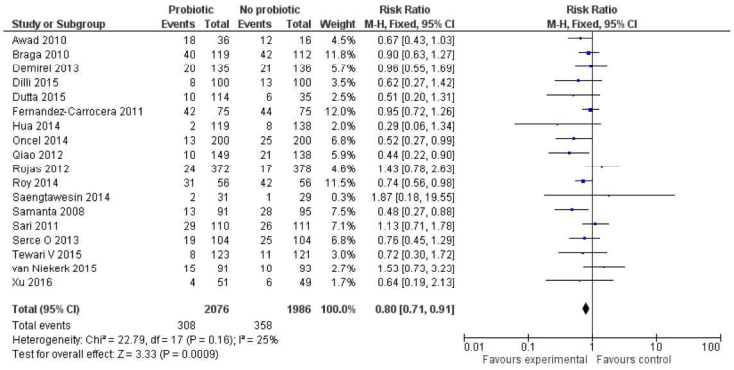
Figure 3

Forest plot: Effect of probiotics on late onset sepsis (LOS)



Forest plot: Effect of probiotics on late onset sepsis (LOS)

297x209mm (300 x 300 DPI)

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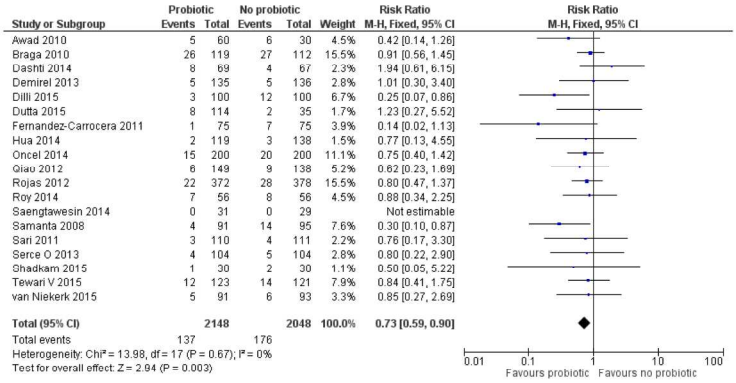
Figure 4

Forest plot: Effect of probiotics on all cause mortality



Forest plot: Effect of probiotics on all cause mortality

297x209mm (300 x 300 DPI)

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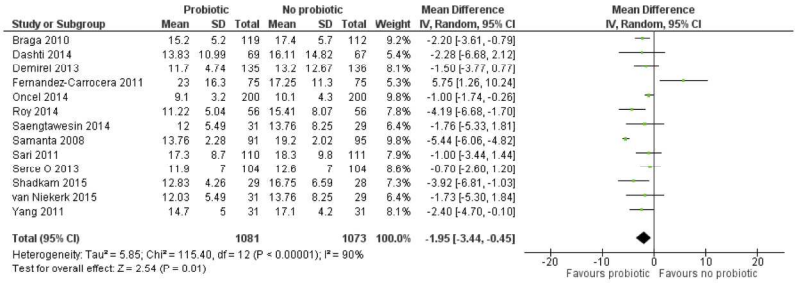
Figure 5

Forest plot: Effect of probiotics on time to full feeds (TFF)



Forest plot: Effect of probiotics on time to full feeds (TFF)

297x209mm (300 x 300 DPI)

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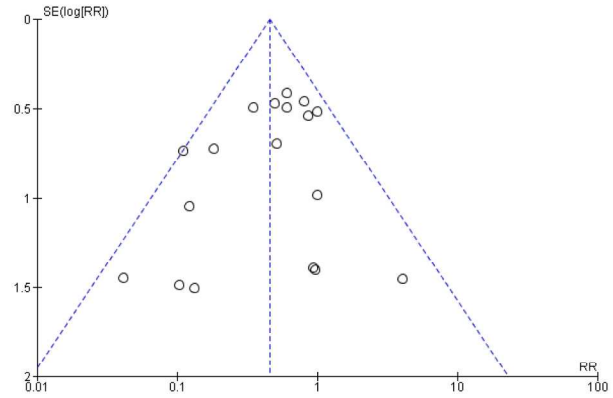
Figure 6
Funnel plot assessing publication bias



Funnel Plot assessing publication bias

297x209mm (300 x 300 DPI)

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Appendix 1: Search strategy

When searched: December 2016 and January 2017

PubMed:

- ((("Infant, Newborn"[Mesh]) OR ("Infant, Extremely Premature"[Mesh] OR "Infant, Premature"[Mesh])) OR ("Infant, Low Birth Weight"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh])) AND "Probiotics"[Majr]: **716**
- (("Infant, Extremely Premature"[Mesh] OR "Infant, Extremely Low Birth Weight"[Mesh] OR "Infant, Very Low Birth Weight"[Mesh] OR "Infant, Small for Gestational Age"[Mesh] OR "Infant, Premature, Diseases"[Mesh] OR "Infant, Premature"[Mesh] OR "Infant, Newborn, Diseases"[Mesh] OR "Infant, Newborn"[Mesh] OR "Infant, Low Birth Weight"[Mesh])) AND (((("Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Saccharomyces"[Mesh]): **774**
- probiotics and preterm infants: **350**
- probiotics and low birth weight infants: **146**
- probiotics and sepsis:**321**
- probiotics and ELGAN(extremely low gestational age) infants: **7**
- probiotics and Necrotizing enterocolitis: **381**

EMBASE: (probiotics.mp. or probiotic agent)/AND **(**preterm infant.mp. OR prematurity/low birth weight infant.mp. OR low birth weight/ very low birth weight infant.mp. OR very low birth weight/extremely low birth weight infant.mp. OR extremely low birth weight/small for gestational age.mp. OR small for date infant OR ELGAN.mp OR extremely low gestational age neonate.mp): **711**

CINAHL: 113

Cochrane: 84 trials

who.int /ictrp (WHO International Clinical Trials Registry Platform): 26, Relevant: 17, Recruiting: 4 (2 of the relevant)

PAS 2000-2014: 187 (probiotics), 68 (Bifidobacteria), 137 (Lactobacillus/ Lactobacilli), Saccharomyces (15)

PAS 2015: 17 (probiotics), 6 (Bifidobacteria), 4 (Lactobacillus/ Lactobacilli), Saccharomyces (2)

Grey literature search: Using term “probiotics and preterm infants”

- **Ntis.gov/:** 42, Relevant: 0; **Opengrey.eu/:** 2, Relevant: 0; **Trove.nla.gov.au**: 495, Duplicates:253, Not relevant:242

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5,6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6,7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6,7 and appendix1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Figure 1
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7,8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7.8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2-5
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9,10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 3,4
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11,12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11,12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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